

ANNUAL CELLULOSE CROP OPTIONS FOR ETHANOL AND OIL CROPPING
INTENSIFICATION FOR BIODIESEL FEEDSTOCKS

by

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Abstract

Ethanol from cellulose and biodiesel are both advanced biofuels according to the renewable fuel standard version two (RFS2) as part of the Energy Independence and Security Act of 2007. Agricultural production of feedstocks for these fuels can occur as co-products from the primary use of the crops. Use of cellulosic material produced from annual grain and sugar crops does not displace land use from grain and sugar production. Production of corn (*Zea mays* L.), grain sorghum, dual purpose forage sorghum, sweet sorghum, and photoperiod sensitive sorghum (*Sorghum bicolor* (L.) Moench) are all primarily driven for products other than cellulosic ethanol. Corn production is driven by grain and silage markets with fodder occasionally used for forage. Grain sorghum production is driven by grain markets and grown primarily in semi arid regions. Dual purpose forage sorghum is used for forage both as baled hay and as silage. Sweet sorghum is produced for sugar and molasses production. Photoperiod sensitive sorghum is produced for baled hay. The current study tests the effect of seeding rate on cellulosic ethanol on each crop. Yellow grease is the most common source of oil for biodiesel production. Intensification of oil crop production may increase the feedstock availability for biodiesel. The current study uses double cropping of spring camelina (*Camelina sativa* (L.) Crantz), spring canola (*Brassica napus* L.), sesame (*Sesamum indicum* L.), safflower (*Carthamus tinctorius* Mohler, Roth, Schmidt and Bourdeux), soybean (*Glycine max* L.), and sunflower (*Helianthus annuus* L.) to search for cropping system options that will produce more oil on an annual basis than full season crops. The full season crop options used were maturity group IV soybean, maturity group V soybean, and full season sunflower. Fertility inputs are inherently less for the non legume crops due to the N fixation ability of symbiotic rhizobium. Canola and camelina are also more sensitive to sulfur deficiency than many crops.

Long chain and polyunsaturated fatty acids have higher market values than biodiesel. Separation of these fatty acids from the lipid profile of oil seed crops provides additional demand for oil seed crops. Demand for the crops will drive commodity prices and move land use into oil crop production. The second year of oilseed production provided an opportunity to look at lipid profiles of successfully produced crops during a drought year.

Three new discoveries were concluded. Grams cellulosic ethanol g⁻¹ stover is not affected by density within the densities considered. Among the double crop options tested only sesame after spring crops was viable in normal years and none were viable in an extreme drought year. Lipid profiles are provided for crops produced in concurrent field growing conditions.

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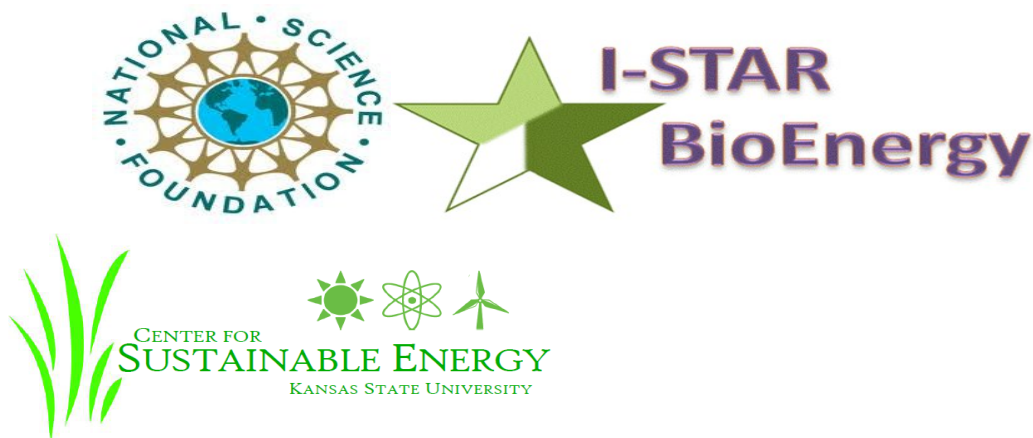
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Preface

Conversion of agricultural products to bio-energy can take many forms. Among the conversion options are ethanol from cellulose and biodiesel from vegetable oils. Cellulosic ethanol can be produced from several sources including lumber waste, forest undergrowth, perennial grasses, and stover from annual starch crops. The first paper in this dissertation focuses on the use of stover from sorghum and corn as a feedstock for cellulosic ethanol. Plant density is reviewed as a potential influence on the production of biomass. Biodiesel production can occur from multiple oil seed crops, dried distiller's grains with solubles, and yellow grease. Increasing annual oil yield from oil seed crops will contribute to the availability of biodiesel if fuel use increase does not exceed biodiesel production potential from the oil yield increase. The second chapter attempts to increase annual oil yield by testing multiple crop combinations for oil yield. Demand for oil seed crops can influence the production of them through increased land use or increased yield through research and development initiatives. Separation of lipids for alternative markets will leave the remaining bulk of oils for use in biodiesel production. The third chapter displays the lipid profiles of soybean, sunflower, camelina, and canola.

Multiple factors exist for density to affect cellulosic ethanol yield per unit of land. In corn an increase in total biomass is beneficial as a density affect when the prior density was below the density to produce maximum grain yield. Increasing density beyond the density to produce maximum grain yield is detrimental due to both loss in grain yield and increased seed cost. Frequently in sorghum, density will not affect either grain yield or biomass production within the range considered in chapter one. Ethanol per unit of stover mass can change through density if the leaf mass to stalk mass ratio changes or if density inhibits maturity. Managing grain yield goals, biomass production, and ethanol yield efficiency from biomass will reveal the density for production of cellulosic ethanol.

Soybean production is a common source for vegetable oil in the Great Plains states, but may not be the highest producer of oil as a feedstock for biodiesel on an annual basis in many field environments. Environments such as alkaline soils and the presence of soybean cyst nematode can harm soybean production. Even in healthy environments other crops or crop combinations may exceed the annual oil production of soybean. Canola is grown in the Great Plains states with the highest concentration of canola production in the northern Great Plains. Canola production is expanding south with breeding efforts in Kansas and Oklahoma. Camelina

can be grown on marginal land where many other crops cannot survive. Sunflower oil is of higher value than biodiesel in many situations, but can be used as yellow grease in biodiesel production. Sunflower production is regarded as drought tolerant, but detrimental to water availability for the following crop. Safflower production is concentrated on land where soybean growth is poor. Sesame production is limited in the United States. Combining two or more of these crops in a single year increases the potential to exceed soybean oil production on an annual basis. A double crop system would have to exceed the soybean oil productivity by enough to cover the increased input costs to be a viable alternative to full season soybean as an oil source. Chapter two compares the oil production of eight double crop options, two full season soybean varieties, and full season sunflower.

Value added products can be more economical to manufacture than biodiesel. Separation of fatty acids from oil seeds enables development of several products with the bulk of the remaining oils available for biodiesel. Saturated fatty acids are used for bread preservatives, and cosmetics. Monounsaturated fatty acids are used for polyesters and drug delivery vehicles. Polyunsaturated fatty acids are used for bio-plastics and nutritional supplements. Profiling vegetable oils for their concentration of fatty acids reveals their niche for value added product development. Chapter three identified the presence and concentration of eighteen lipids in soybean, sunflower, canola, and camelina. Five of the lipids identified were considered feasible for separation in industrial applications due to their concentration and previous use.

Cumulatively the papers represent cellulosic ethanol production techniques from a field perspective, an attempt to increase biodiesel production beyond full season soybean feedstock production, and use of high value lipids to move biodiesel to the status of a co-product to value added products.

Chapter 1 - Biomass and Grain Yields as an Ethanol Feedstock from Sorghum and Corn as Affected by Seeding Rates

ABSTRACT

Ethanol from sorghum (*Sorghum bicolor* (L.) Moench) is considered an advanced biofuel as defined by USDA, 2012 if the cellulosic biomass is used. Starch based ethanol from sorghum grain is considered an advanced biofuel by the modified renewable fuel standard in the Energy Independence and Security Act of 2007 (RFS2) if the carbon dioxide (CO₂) equivalent greenhouse gas emissions are less than petroleum greenhouse gases were in 2005. A two year sorghum field study was conducted in KS to determine the relationship between seeding rates and biomass produced from four cultivars, ‘Pioneer 84G62’ grain sorghum (Pioneer Hi-bred, Johnston, IA), ‘NK300’ dual purpose forage sorghum (Sorghum Partners, New Deal, TX), ‘M-81E’ sweet sorghum (Miss St. Univ, Miss.St., MS), and ‘PS1990’ photoperiod sensitive sorghum (Sorghum Partners, New Deal, TX). The seeding rates recommended by Kansas State University Extension for grain sorghum were used as a baseline seeding rate (R). Each sorghum variety was planted at 0.5R, R, and 1.5R. Sweet sorghum and photoperiod sensitive sorghum produced the most biomass. The highest grain yields varied with location. Total cellulosic material varied by location year and variety.

Corn (*Zea mays* L.) stover is considered an advanced biofuel by RFS2 (USDA 2012). A one year field study was conducted in KS to apply classic corn yield models to KS environments and provide feedstock for cellulose fermentation. Corn was planted at rates of no competition, 0.5R, R, 1.5R, and 2R using ‘Pioneer 33B54’ (Pioneer Hi-bred, Johnston, IA) in Tribune and ‘DKC-63-42’ (DeKalb, DeKalb, IL) in Manhattan. At both locations grain yield followed the pattern of the Duncan yield model. Total aboveground biomass reflected a rectangular hyperbola pattern.

Fermentation of cellulosic material showed differences in ethanol yield between cultivars and years for sorghum and between populations for corn. High population sorghum contains more leafy material at maturity and can prevent reaching physiological maturity at the end of the growing season. Sweet sorghum had the highest ethanol yield. Glucose left in bagasse after juice

extraction created the higher ethanol yield in sweet sorghum. Plant density did not have an effect on ethanol yield. In year two, plants were exposed to a higher osmotic stress resulting in higher tiller count from poor stand establishment. Corn was the only grown in year one. Anecdotal observations showed changes in the corn ring to pith ratio with changes in density. High density corn had a relatively high rind to pith ratio. Corn without competition mimicked high population corn in this manner as the tillers have similar ratios rind to pith ratios as high population corn. Rind material has more lignin than pith material. This difference resulted in higher cellulosic ethanol yield from corn planted at 74,000 plants ha⁻¹.

INTRODUCTION

Interest in cellulosic crop production has increased due to the Energy Independence and Security Act of 2007. Commercial use of cellulosic material for bioenergy is limited at this time. Abengoa Bioenergy (Seville, Spain) is producing advanced biofuels in York, NE and Salamanca, Spain. Cellulosic ethanol is being produced at a pilot scale in St. Joseph, MO by ICM engineering (Colwich, KS) . Florida Crystals (South Bay, FL) is utilizing cellulosic material to produce electricity from direct combustion. Pyrolysis (Wang, et. al 1997) and gasification (Lv, 2007) are also options to convert cellulosic material to bioenergy. Current commercial production of cellulosic ethanol falls well short of RFS2 incentives (EPA, 2012)

Cost of biomass production and delivery changes with time and species. Corn stover delivered to ethanol plant costs were \$80.30 Mg⁻¹ in February 2012 and switchgrass delivered cost were \$79.30 Mg⁻¹. Sorghum stover costs were similar to corn (Gonzalez et. al, 2012). Break even farm gate value estimates for switchgrass biomass varied from \$39.48 Mg⁻¹ to \$46.62 Mg⁻¹ (2007 dollars) (Popp and Hogan 2007). Land area planted to sorghum in Kansas may increase with the depletion of Ogallala aquifer resources. Use of grain sorghum stover for cellulosic feedstock removes less land from food production than a perennial dedicated energy crop. The differences in biomass production between tall sorghums (sweet and photoperiod) and grain sorghum may justify production of these feedstocks in lieu of grain sorghum. Dual purpose forage sorghum can produce higher grain yields than grain sorghum in wet environments (Katayama 1967). Sorghum plants up to 2.5 m tall create a larger source for grain assimilates than short grain sorghums. Grain harvest of dual purpose forage sorghum is not feasible with current technology.

Sorghum is a relatively management insensitive crop. Tall sorghums have been reported to be insensitive to population (Dooley, 2010). Increasing nitrogen (N) rates beyond 150 kg ha⁻¹ did not increase yield (Clegg, 1982). Sorghum has been reported to respond less to irrigation above 20.6 cm year⁻¹ than corn (Stone, 1996). Kansas State Research and Extension seeding rate recommendations increase with increases in expected annual rainfall for the region planted.

Tillering tendencies offer an opportunity to manage ethanol outputs. The number of tillers per plant decreased as population increased (Lafarge et. al, 2002). Early season crowding suppresses tillering potential. Tillers can often have delayed development increasing the potential to not achieve physiological maturity when the main stalk is harvested. This difference in physiological maturity creates the potential for differences in cellulosic ethanol production from plants with tillers. Plants grown at higher plant densities also have been reported to have a higher leaf mass to stalk mass ratio (Worley et al., 1990) These changes also create potential biofuel yield differences because leaf and stover compositions differ.

The distributions of yield components in sorghum can be influenced by population. An interaction effect has been reported between grain sorghum harvest indices, genotype, and the date of planting (Hammer and Broad, 2003). Maturity can also be delayed at excessive populations. This delay can prevent sweet sorghum from reaching the soft dough stage prior to the first frost in temperate latitudes. Photoperiod sensitive sorghum yield component distribution is less likely to be affected as first frost occurs shortly after daylight recedes to less than 12.5 hours day⁻¹ in Kansas, the trigger required to induce floral initiation.

Total aboveground biomass yields have been shown to follow a rectangular hyperbola model in multiple crops with respect to population. A rectangular hyperbola model (equation 1)

$$M = P * (AP + B)^{-1} \quad [\text{eq. 1}]$$

was first demonstrated for aboveground biomass in Japan (Shinozaki and Kira 1956). M is the total biomass ha⁻¹; P is the plant density; and A and B are coefficients defined by genotype, environment and their interaction. This model has been reported to have higher correlations to biomass yield than the Mitscherlich equation for crops with harvest intervals longer than six weeks (Overman and Scholtz III, 2002). The model has been applied to corn by Russell (1979) and Ballard et al., (2008). A rectangular hyperbola can be transformed to a linear form as the inverse of mass per plant or biomass plant density with the units kg plant⁻¹. Biomass plant density is expressed as m⁻¹ (equation 2).

$$m^{-1} = AP + B \quad [\text{eq. 2}]$$

The reciprocal of these data can be graphed linearly as well, but is not expressed linearly relative to the rectangular hyperbola model (equation 3).

$$m = (AP + B)^{-1} \quad [\text{eq. 3}]$$

Applying a rectangular hyperbola to sorghum plant density can result in two distinct interpretations. The initial population is the emerged stand. Early in the growing season sorghum culm (stalk) numbers can increase through field through tiller production. The end of season stalk count is the total of all tillers and main culms.

Individual corn grain yields decay exponentially in response to density increases (Duncan, 1958). Yields display this response with the model (equation 4).

$$Y = aPe^{-bP} \quad [\text{eq. 4.}]$$

The population which produces maximum yield P_{\max} occurs at $P = b^{-1}$. This relationship enables producers to determine P_{\max} using yield plant⁻¹ (y) (equation 5).

$$y = YP^{-1} = ae^{-bP} \quad [\text{eq. 5}]$$

Regression of equation five is available in Microsoft Excel, so the purchase of more specialized regression software is not required for producers to use the Duncan yield model.

The impact of increasing culm number, either by seeding rate decisions or plant responses via leaf and stalk material production may not only affect biomass yield, but may also influence bioenergy production from the biomass. As a result we had four objectives. 1. Determine if initial or end of season plant density effects on corn and sorghum biomass and grain yields are best described by a rectangular hyperbolic equation. 2. Determine plant density effects on the distribution of yield components in sorghum and corn. 3. Determine if differences exist in cellulosic ethanol yield between cultivars. 4. Determine the effects of plant density and cultivar on final ethanol production ha⁻¹.

MATERIALS AND METHODS

Field studies were established at Garden City, Manhattan, and Tribune, Kansas in 2009 and Manhattan and Hutchinson, Kansas in 2010. Plant density treatments were established using the seeding rate settings of a John Deere 7200 series no till planter modified into a two row plot planter. Sorghum seeding rates were based on the Kansas State Research and Extension Grain

Sorghum Production Handbook (Vanderlip et al., 1998). In Manhattan (39.19° N, 96.60° W) seeding rates were 148,000 seeds ha⁻¹ (R), 0.5 R and 1.5 R. The soil type in Manhattan was a Bellevue silt loam (sandy over loamy, mixed, mesic Type Endoaqualls) in 2009 and a Rossville silt loam (fine-silty, mixed, superactive, mesic Cumulic Hapludoll) in 2010. In Hutchinson (38.06° N, 97.91° W) the seeding rates were 111,000 seeds ha⁻¹ (R), 0.5 R and 1.5 R. The soil type in Hutchinson was a Farnum clay loam (fine-loam, mixed, superactive, mesic, Pachic Argiutolls). In Garden City (37.97° N, 100.87° W) the seeding rates were 59,300 seeds ha⁻¹ (R) and 1.5 R. The low population was achieved in Garden City by manually thinning after planting at R. The soil type in Garden City was a Richfield and Ulysses complex soil (fine, smectitic, mesic Aridic Argiustolls). Corn was planted at a rate of 69,000 seeds ha⁻¹ (R), 0.5 R, 1.5R, 2R and no competition were established in Manhattan. No competition plots were a single corn plant isolated in an area that was 3 m². The soil type for Manhattan corn was a Belleville silt loam and in Garden City the soil type was a Ulysses silt loam (fine-silty, mixed, superactive, mesic Aridic Argiustolls) In Garden City R for corn was 29,700 seeds ha⁻¹. Corn was not planted at 0.5 R in Tribune. Border rows were not planted between populations. Samples were harvested from the middle two rows.

Sorghum

A randomized complete block design with four replications was used in year one. Modifications were made to the experimental design in year two due to concerns of shading by the tall sorghums on the two shorter cultivars. No detrimental shading effect was observed. A split plot design with cultivar as the main plot and population as the sub plots was used in 2010. Four border rows of grain sorghum were planted between cultivars to reduce the risk of a shading effect on the neighboring treatments. Sorghum was planted on 22 May 2009 in Manhattan 26 May in Garden City. A pre-emergence Roundup Weathermax (1.06 kg a.i. glyphosate ha⁻¹) treatment was applied both years. In 2009 residual weed control was achieved with 4.9 liters ha⁻¹ of preplant Bicep II Magnum (1.9 kg ha⁻¹ a.i. atrazine and 1.5 kg ha⁻¹ a.i. S-metolachlor). A preplant burn down treatment of 2.3 liters hectare⁻¹ of glyphosate (1.54 kg a.i. ha⁻¹) and 1.2 liters ha⁻¹ of 2,4-D (0.87 kg a.i. ha⁻¹) was applied 10 days before planting. Planting occurred on 25 May 2010 in Manhattan and 29 May in 2010 in Hutchinson. In 2010, the plots were manually cleaned three times to remove emerged Palmer amaranth (*Amaranthus palmeri*

S. Wats.). Nitrogen was applied fifteen to twenty days after planting using urea at a rate of 112 kg N ha⁻¹. Soil test P, K, and pH were within optimum ranges for sorghum production with the exception of Garden City in 2009. Ammonium phosphate was added on the day of planting at a rate of 28 kg ha⁻¹ of P₂O₅ in Garden City. The post emergence N rates were adjusted to account for the ammonium phosphate application.

Harvest was completed separately for grain, sweet juice, and cellulosic material. Grain harvest was accomplished by hand pruning heads from the grain sorghum and dual purpose forage sorghum from the center two rows with a 7.0 m² sample of four row plots in Manhattan, and Hutchinson and six row plots in Garden City. Grain moisture was measured using a Dickey John GAC 2000 moisture reader. Grain yield was adjusted to standard market moisture of 0.14 g water g⁻¹ grain. Sweet sorghum juice was extracted by crushing ten plants in each plot using a three roll press. Biomass was removed in bulk for the same 7.0 m² area using a silage chopper after the completion of grain and juice samples. A 100 – 200 liter subsample of biomass was weighed before and after drying at 60⁰ C to project the total dry matter. Bagasse and non juiced sweet sorghum stalks were both retained for the fermentation study. Stover subsamples were dried at 60⁰ C for moisture adjusted yield.

Stand counts were taken 20 days after planting to assess the initial plant density. End of season stalk counts were projected as a ratio of the mass of 7.0 m² to the mass of ten plants. The linear form of a rectangular hyperbola is expressed as the inverse of individual plant mass or biomass plant density using equation [1]. The independent variable for the regression analysis are expressed as stalks kg⁻¹ instead of the traditional kg stalks⁻¹. B is the asymptotic limit to the plants' biomass in the given field conditions. This allows for the estimation of the values A and B for the lines. Sigma Plot 11 (Systat Software, San Jose, CA) was used to complete the regression. Correlation significance was lock design tested using R². Differences in grain yield and juice yield were evaluated using analysis of variance F test with the help of the PROC-MIXED function in SAS 9.3.

Corn

A randomized complete block design with four replications was used. Weed management was accomplished using preemergence glyphosate at a rate of 1.06 kg a.i. ha⁻¹ and preemergence Lumax (7.51 kg a.i. S-metolachlor ha⁻¹, 0.300 kg a.i. mesotrione ha⁻¹, and 1.12 kg a.i. atrazine).

Planting occurred on 11 May 2009 in Manhattan and 15 May 2009 in Tribune. Post emergence spot application of glyphosate was also used. Nitrogen was applied 15-20 days after planting with urea at a rate of 168 kg N ha⁻¹. P, K and pH soil tests were within optimal range.

Stand counts were taken 15-20 days after planting to determine the independent variable for regression. The stover of five plants for each harvested grain sample was used to project the plot mass.

Harvest occurred following physiological maturity. Ears were removed from two 3.5 m² areas in each plot from the middle two rows of four row plots. A stationary sheller separated and cleaned the grain. Grain moisture was measured using a Dickey John DAC 2000 moisture tester. Grain yield was adjusted to 0.155 g water g⁻¹ grain moisture. Ten plants from within the area were cut at the soil surface for biomass samples. Biomass samples were dried at 60⁰C to evaluate stover yield. Stover yield was expressed as dry matter. Stover samples were retained for ethanol fermentation. Table 1.1 shows the characteristics of each corn and sorghum cultivar.

Regression analysis for the Duncan grain yield model and Russell biomass model was accomplished using Sigma Plot 11. R² was used to test the significance of these curve fits.

Fermentation

Stover samples were processed into ethanol to determine if plant management and environmental conditions affected the conversion process. Biomass samples were ground to 200 µm. A 15 g subsample was mixed with a 0.02 ml H₂SO₄ 0.98 ml water solution. This mixture was then autoclaved at 121⁰ C for 30 minutes. Sulfur was removed by rinsing for one minute through a screen. This acid hydrolysis broke open the plant cell walls to allow for enzyme hydrolysis to break down the cellulose into monosaccharides. The samples were dried overnight at 60⁰C. Five grams of the acid hydrolysis treated biomass was mixed with 48 ml of citric acid pH 5.0 (50 mM citrate buffer). The mixture was autoclaved at 121⁰C for fifteen minutes.

Enzyme hydrolysis broke down the cellulose into yeast accessible six carbon sugars. The enzymes added to the biomass and citric acid were 1.25 ml of cellulase (Novozyme 22074, Novoyme, Bagsvaerd, Denmark) and 0.71 ml of glucosidase (Novozyme 50010). The enzyme hydrolysis occurred over 72 hours at 40⁰C in an orbital shaker at 30 rpm. The solids from enzyme hydrolysis were separated using a centrifuge at 10,000 rpm for twenty minutes. A one

ml sample of the liquid was saved for high performance liquid chromatography (HPLC) analysis. The remaining liquids were used for fermentation.

Fermentation required the input of additional nutrients and yeast. We added 0.6 mg $(\text{NH}_4)_2\text{SO}_4$, and 0.9 mg yeast extract to the enzyme hydrolysis liquid. The mixture was autoclaved for fifteen minutes at 121°C . After the autoclaved mixture cooled to room temperature, three ml of yeast broth were added. Fermentation occurred at 35°C in an orbital shaker for fifteen hours. A one ml sample of the fermented liquid was saved for HPLC analyses. HPLC analyses were performed using a mixture of 10% sample and 90% de-ionized water to reduce column clogging potential of water soluble solids within the vial sample. Samples were filtered using $0.45\ \mu\text{L}$ membranes. Placement of samples for HPLC analysis was into a autosampling tray (Prominence, SIL-20AC). Sugar concentration analysis was completed using the binary HPLC system (Shimadzu Scientific Instruments, Columbia, MD) using the Refractive Index (RI) detector (RID-10A) and carbohydrate detection column (300 X 7.8 mm, Phenomenex, USA). Deionized water was collected from the Mill Q (Direct !, Millipore Inc, Billerica, MA), degassed using ultrasonicator (FS 60, Fisher Scientific, Pittsburg, PA) and was used as a mobile phase. Column temperatures were maintained at 80°C , RID at 65°C using a Prominence CTD-20A column oven. The mobile phase was pumped through the column at $0.6\ \text{ml min}^{-1}$. A Na^+ column was used to evaluate the alcohol concentration in each sample.

Any remaining biomass material from the acid hydrolysis was used to evaluate the pre-enzyme hydrolysis neutral detergent fiber (NDF), acid detergent fiber (ADF), and non acid soluble fiber (AD) of each sample. The neutral detergent fiber was the simple sugar content of the samples. The acid detergent fiber was the cellulose and hemi cellulose. The non acid soluble fiber was the lignin content of the samples. A pH of four was used to determine the acid detergent fiber. Enzyme hydrolysis and fermentation results were then coupled with the ADF and NDF results to determine the fermentation efficiency.

Stover yields from each field treatment and final ethanol concentrations were used to determine the cellulosic ethanol yield ha^{-1} .

Table 1.1 Cultivar Description for Sorghum and Corn

Cultivar	Species	Maturity	Height	Primary Use
84G62	Sorghum	72 days to flowering	less than 1 m	grain
NK300	Sorghum	medium early	2 m	forage
PS1990	Sorghum	flowers at 12.5 hours of daylight	greater than 3 m	forage
M 81E	Sorghum	late	greater than 3 m	sugar/molasses
33B54	Corn	113 days to physiological maturity	1.5 m	grain
DKC 63-42	Corn	113 days to physiological maturity	1.5 m	grain

Sources: Broadhead, 1991, Pioneer, Sorghum Partners, and Dekalb seed guides

RESULTS AND DISCUSSION

Growing conditions varied between location and year. In 2009 the month of May at Manhattan was dryer than the ten year average (Table 1.2). This coincides with sorghum stand establishment time period. Average rainfall was received the rest of the growing season. Manhattan received near mean rainfall for the summer of 2010. A high wind event resulted in extensive lodging to the Manhattan sweet sorghum and photoperiod sensitive sorghum in 2010. In 2009 at Garden City, less than average rainfall was received at the beginning and end of the growing season. At Hutchinson higher than normal rainfall was received in 2010 except for in September. At Tribune higher than normal rainfall was received in 2009 during June, July, and August. The early growth period of May and end of the growing season were drier than normal.

Yields

Sorghum

Sorghum biomass, grain yield, stover yield, and juice yield were all related to emerged density and end of season stalk density using the Shinozaki and Kira biomass model [eq. 1] and linear models for all sorghum cultivars. Density was also related to end of season stalk density using linear regression. Eight relationships out of the 86 relationships evaluated were significant. No plateaus were reached. In 2009, six significant relationships were found. Manhattan dual purpose sorghum stalk density was significantly related to both a linear model and a hyperbolic model (Fig 1.1). The linear model provided a better fit; $Y = 0.0246T + 7.27$ expressed in $Mg\ ha^{-1}$. In Garden City sweet sorghum juice yield responded negatively to stalk density with linear model; $J = -0.0189T + 7.94$ (Fig. 1.2). Manhattan sweet sorghum juice yield responded positively to stalk density with the linear relationship; $J = 0.19 T + 18.9$ (Figure 1.3). In Garden City juice yield responded with a negative slope to emerged density; $J = -0.024P + 8.92$ (Fig. 1.4). Sweet sorghum stalk density increased with emerged population in Garden City; $T = 0.787P + 8.72$ (Fig. 1.5). T represents stalk count and J represents juice mass.

In 2010, no responses to emerged density or stalk density were significant in Manhattan and three were in Hutchinson. Juice yield increased with tiller density in Hutchinson with the response $J = 0.043T + 2.99$ (Fig. 1.6). Stalk density increased as a function of emerged density

with the response $S = 0.597P + 44.0$ (Fig. 1.7). Grain sorghum stalk density also increased with emerged density with the response $S = 0.538P + 6.82$ (Fig. 1.8).

1.2 Monthly mean temperatures and rainfall totals in 2009 and 2010 for Manhattan

	Monthly average air temperature									Monthly precipitation totals								
	Manhattan			Garden City		Tribune		Hutchinson		Manhattan			Garden City		Tribune		Hutchinson	
	'09	'10	Avg	'09	Avg	'09	Avg	'10	Avg	'09	'10	Avg	'09	Avg	'09	Avg	'10	Avg
	----- ⁰ C-----									-----mm-----								
May	18	17	18	16	16	16	15	18	18	12	92	91	41	74	2.3	7.1	122	97
June	24	25	25	22	22	21	22	26	26	207	168	168	80	76	7.5	6.7	199	109
July	23	27	27	25	26	24	24	27	27	128	106	107	66	64	8.3	7.9	155	89
Aug	23	27	27	23	24	23	23	27	27	135	81	89	46	64	6.8	5.3	100	79
Sept	18	21	22	19	20	17	18	22	22	46	76	69	33	56	2.9	5.3	33	84

Source: Kansas St. Univ. Weather Data Library

Avg represents 30 year mean observations

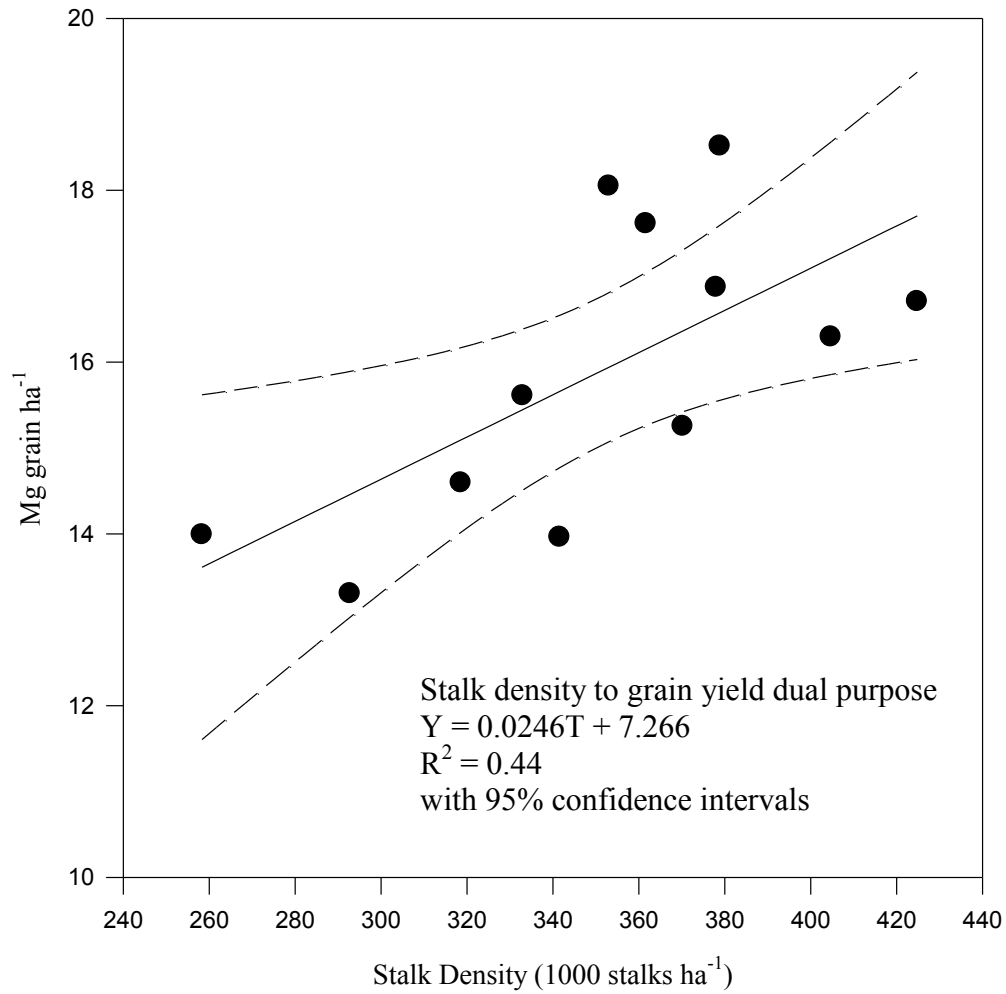


Figure 1.1. Stalk density relationship to grain yield for dual purpose sorghum in Manhattan, KS in 2009. Each replication had a unique stalk count.

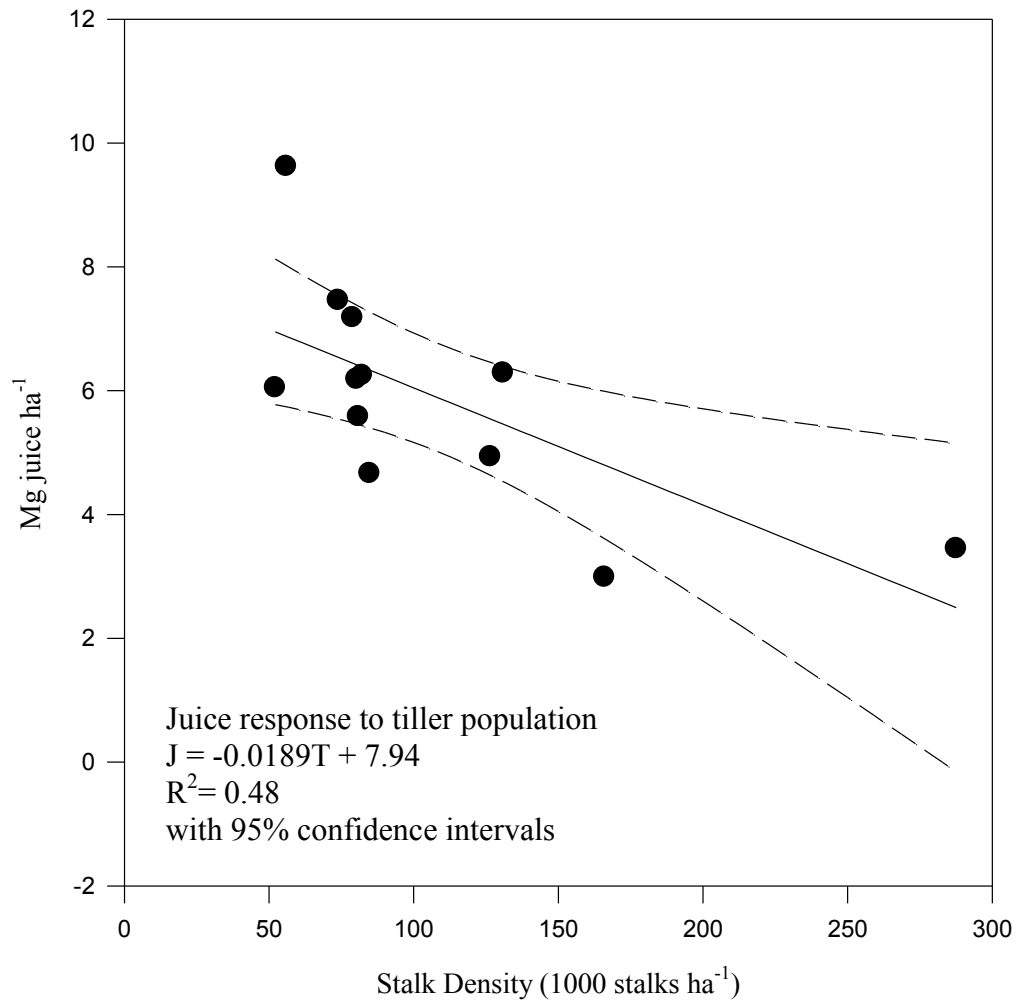


Figure 1.2 Sweet sorghum juice response to stalk density in Garden City, KS in 2009. Each replication had a unique stalk count.

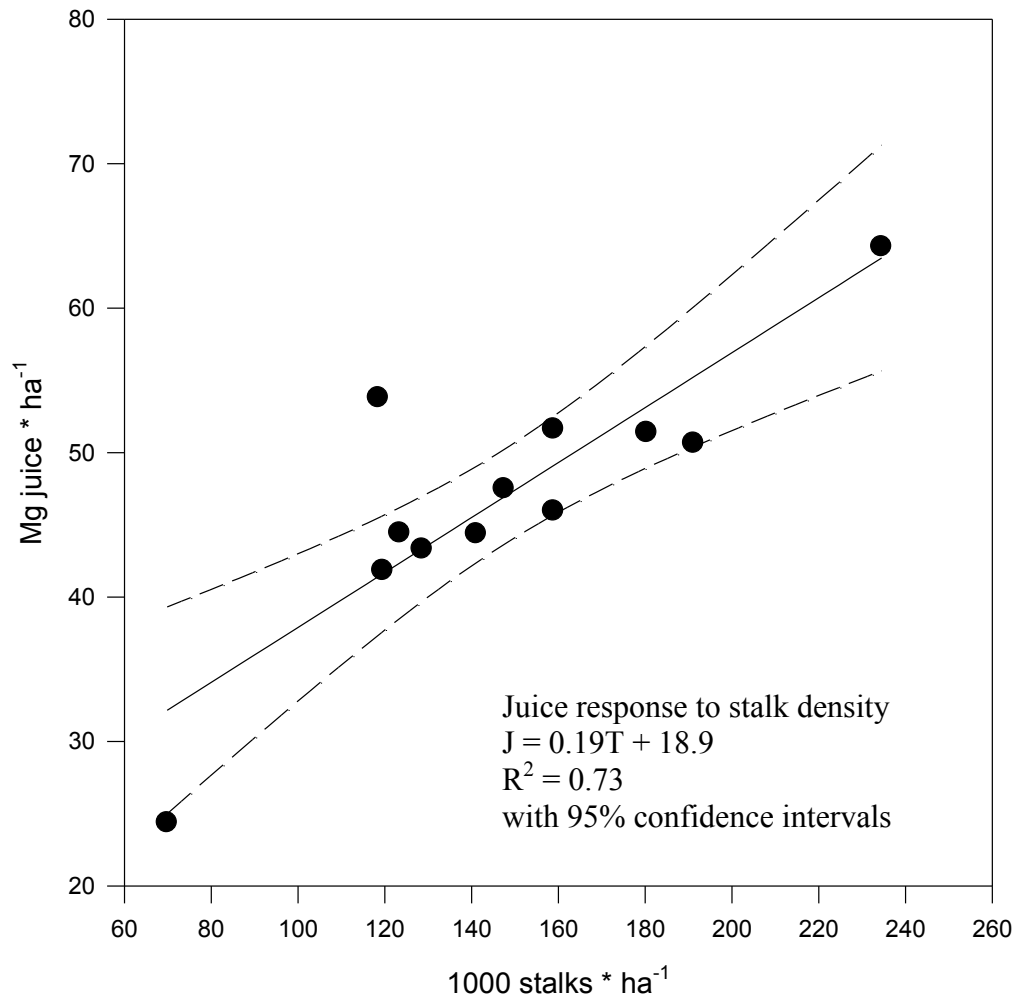


Figure 1.3 Sweet sorghum juice response to stalk density in Manhattan, KS in 2009 Each replication had a unique stalk count.

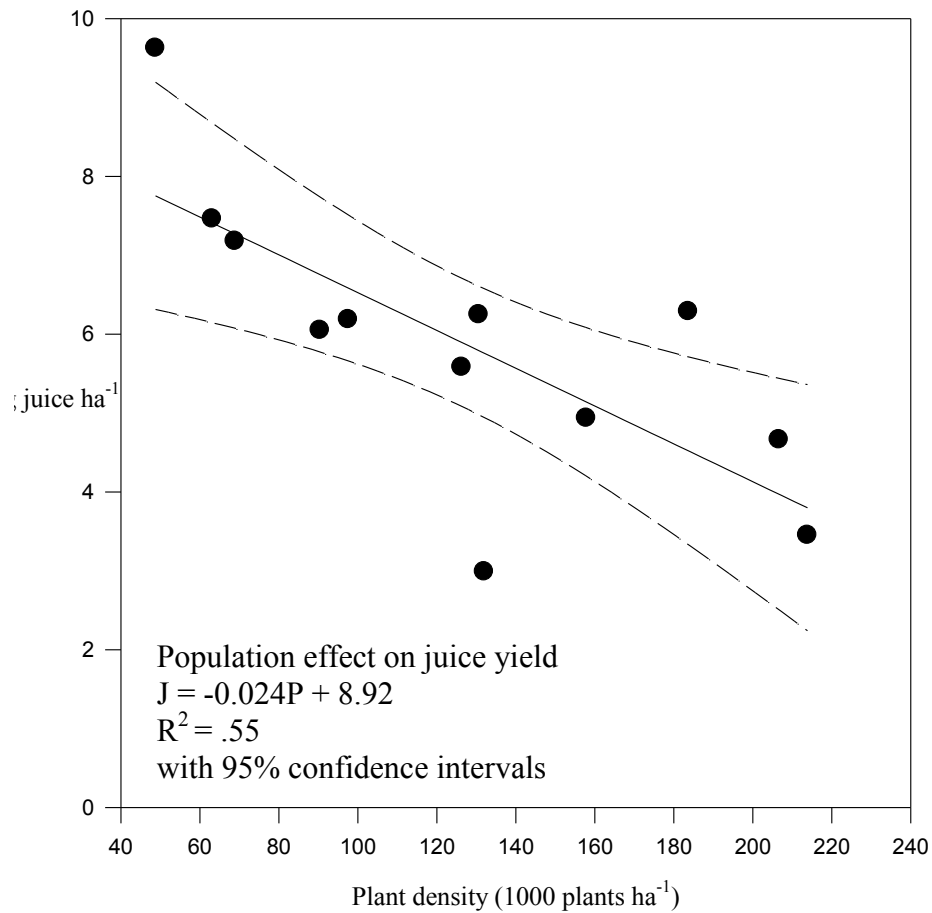


Figure 1.4 Density effect on sweet sorghum juice yield in Garden City, KS in 2009. Each replication had a unique emergence rate. Plant densities higher than seeding rate represent excess seed planted using grain sorghum seed plates with sweet sorghum seed.

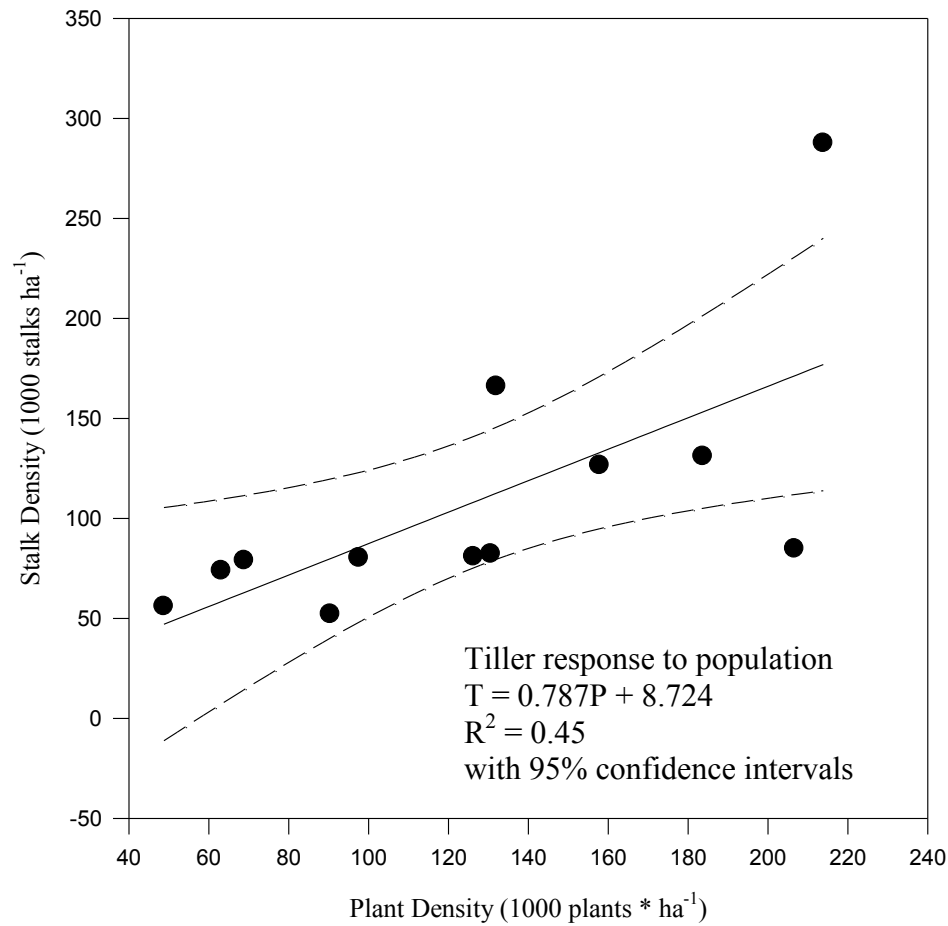


Figure 1.5 Tiller effect on sweet sorghum population in Garden City, KS in 2009. Each replication had a unique emergence rate. Densities higher than the seeding rate represent excess seed loading in planting plate due to smaller seed size of M 81E than grain sorghum seed size.

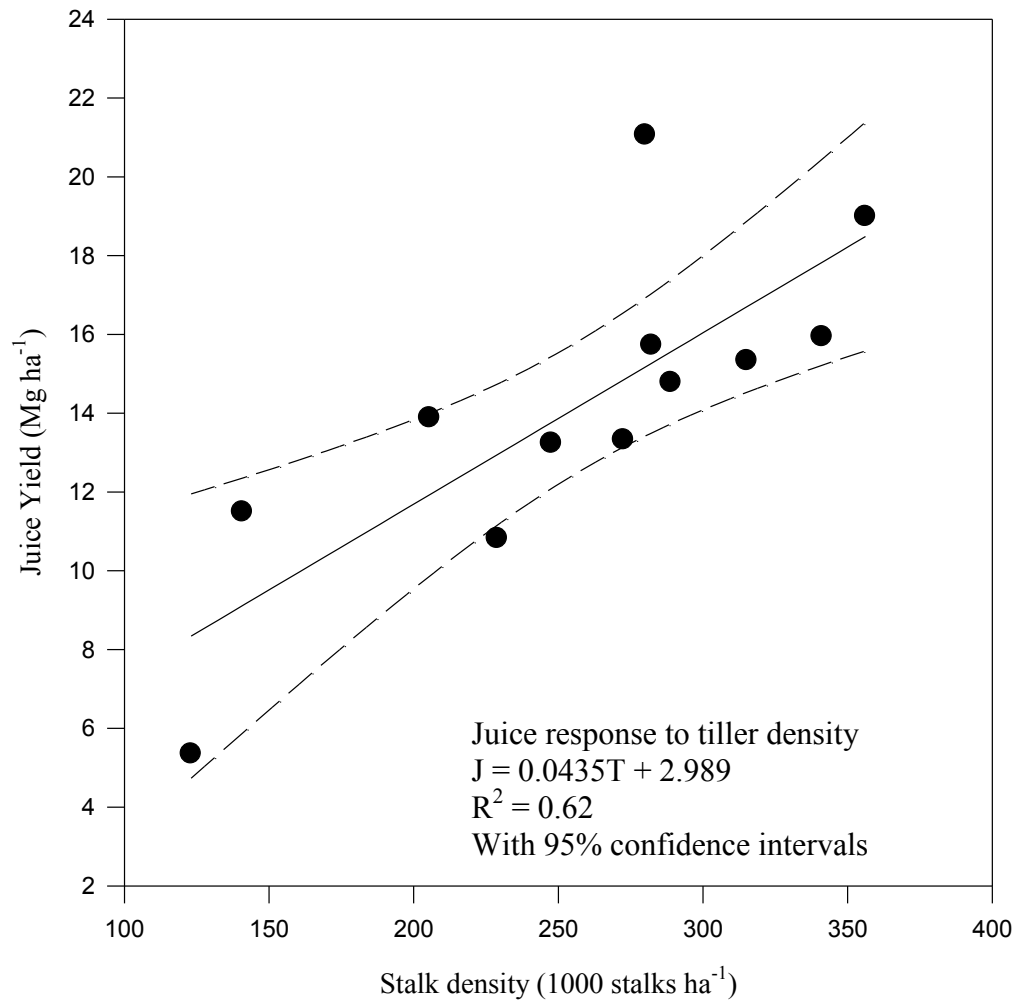


Figure 1.6 Sweet sorghum juice response to stalk density in Hutchinson, KS in 2010. Each replication had a unique stalk count.

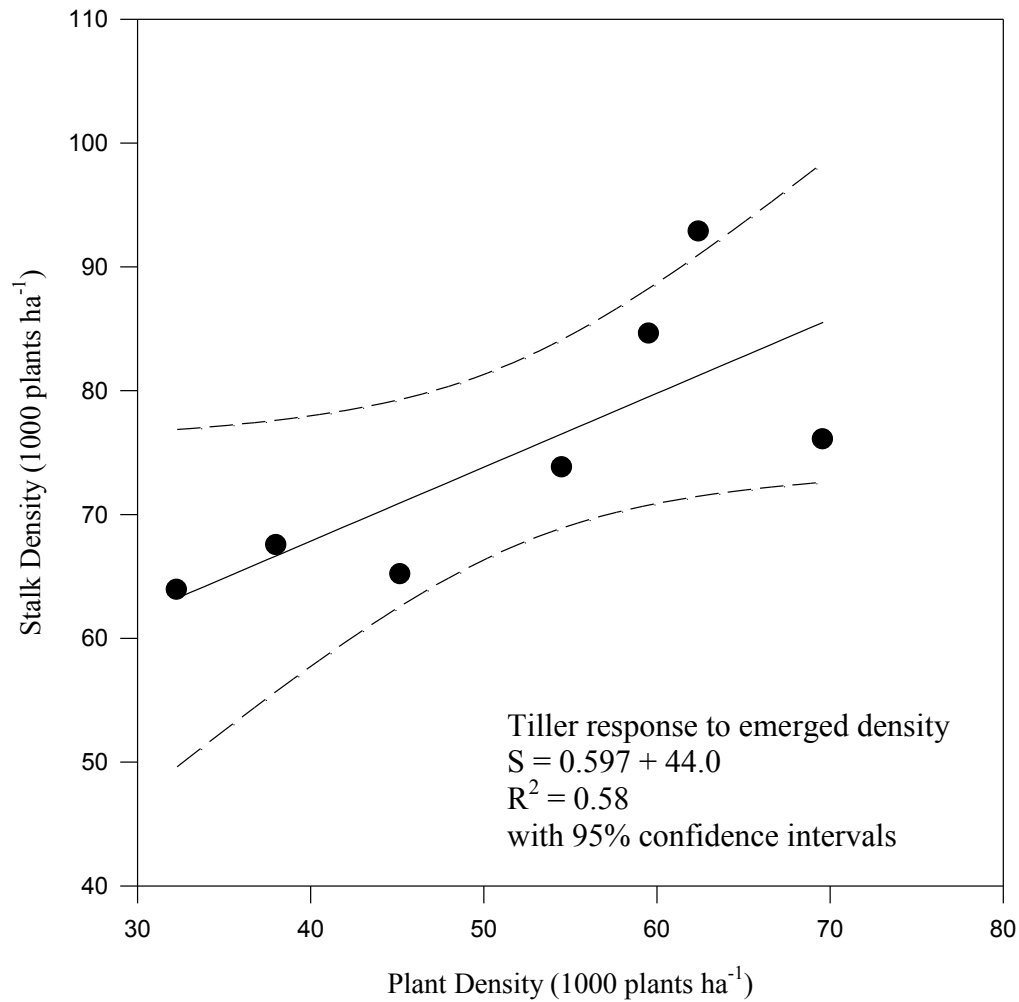


Figure 1.7 Photoperiod sorghum stalk density response to plant density in Hutchinson, KS in 2010. Each replication represents a unique emerged density.

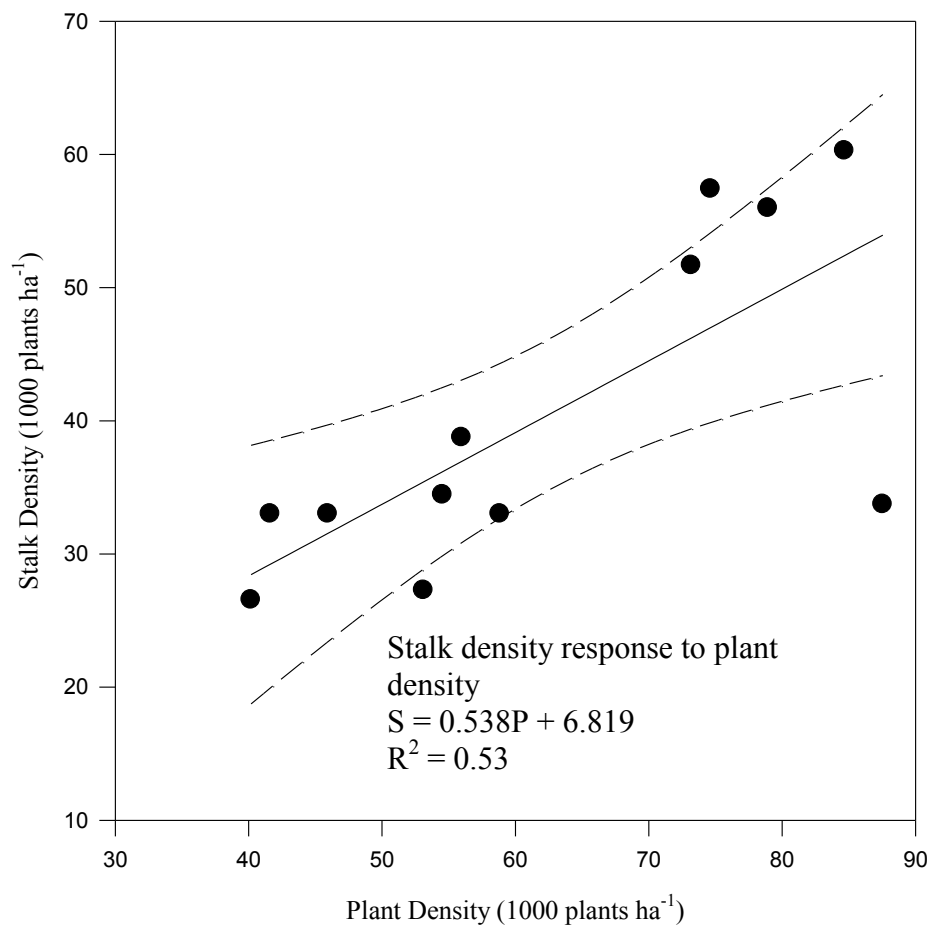


Figure 1.8 Grain sorghum plant density effect on stalk density in Hutchinson, KS in 2010.
Each replication represents a unique emergence rate.

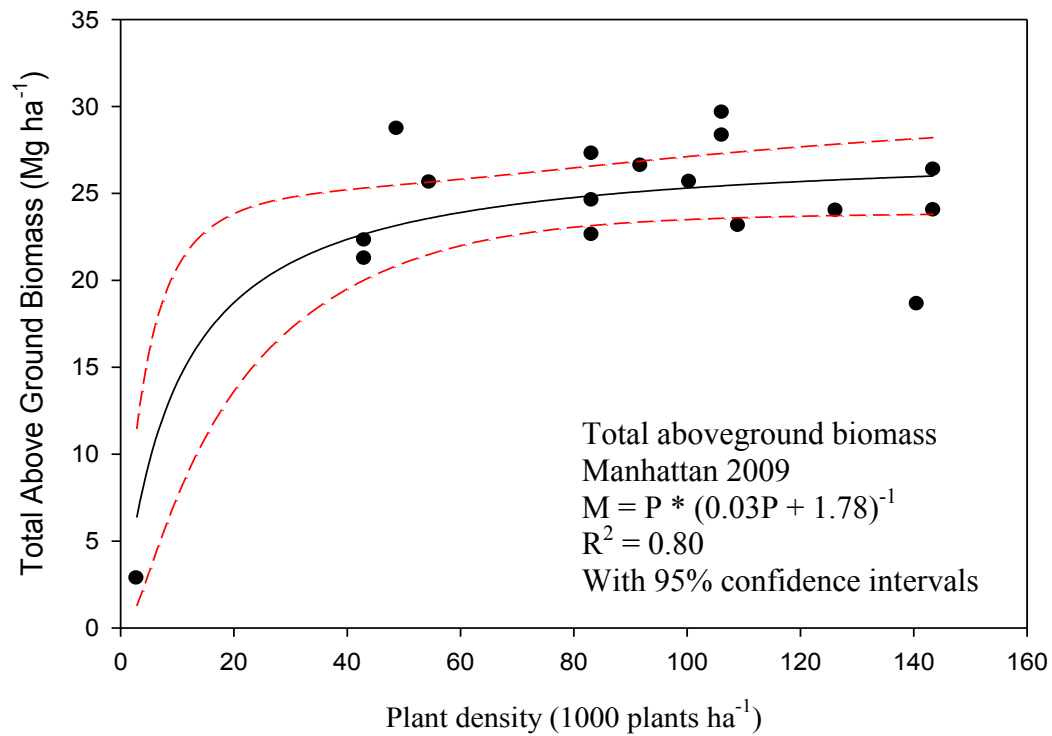


Figure 1.9 Corn total above ground biomass fit to Russell biomass equation in Manhattan, KS in 2009. Each replication represents a unique emerged population.

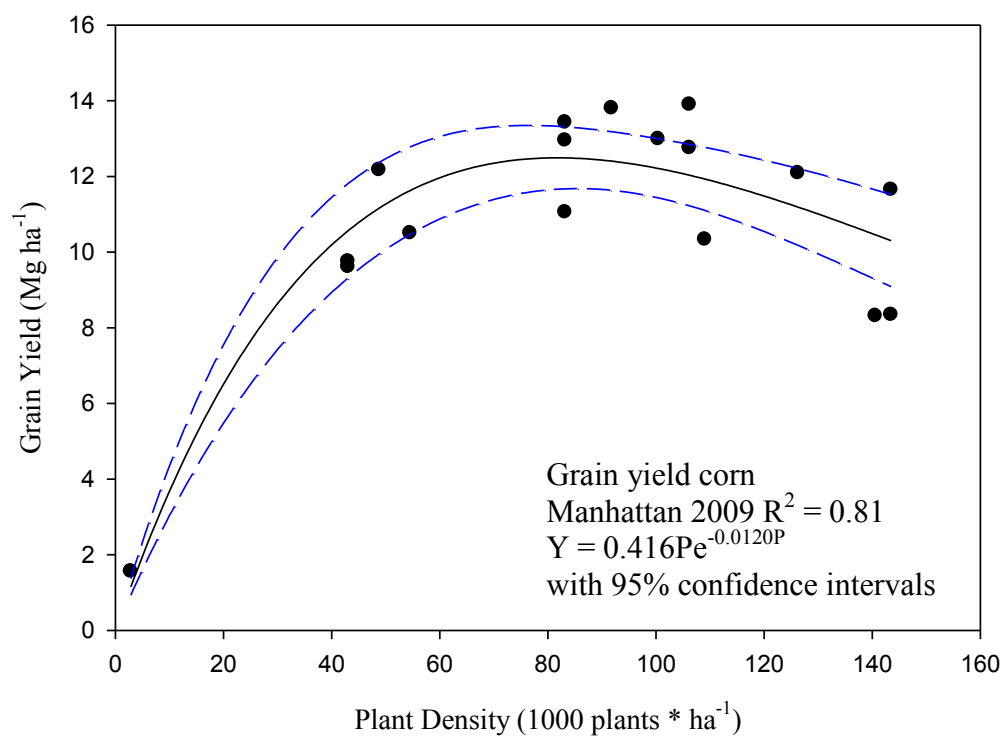


Figure 1.10 Corn grain yield response fit to Duncan yield equation in Manhattan, KS in 2009. Each replication represents a unique emerge population.

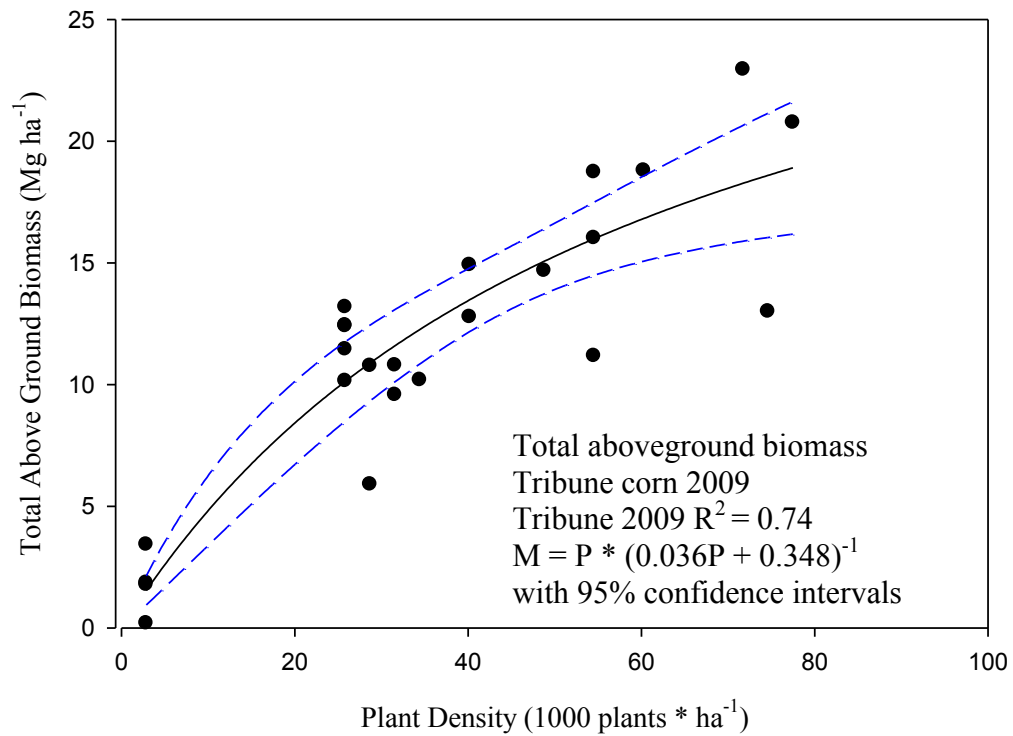


Figure 1.11 Corn total above ground biomass fit to Russell biomass equation in Tribune, KS in 2009. Each replication represents a unique emerged population.

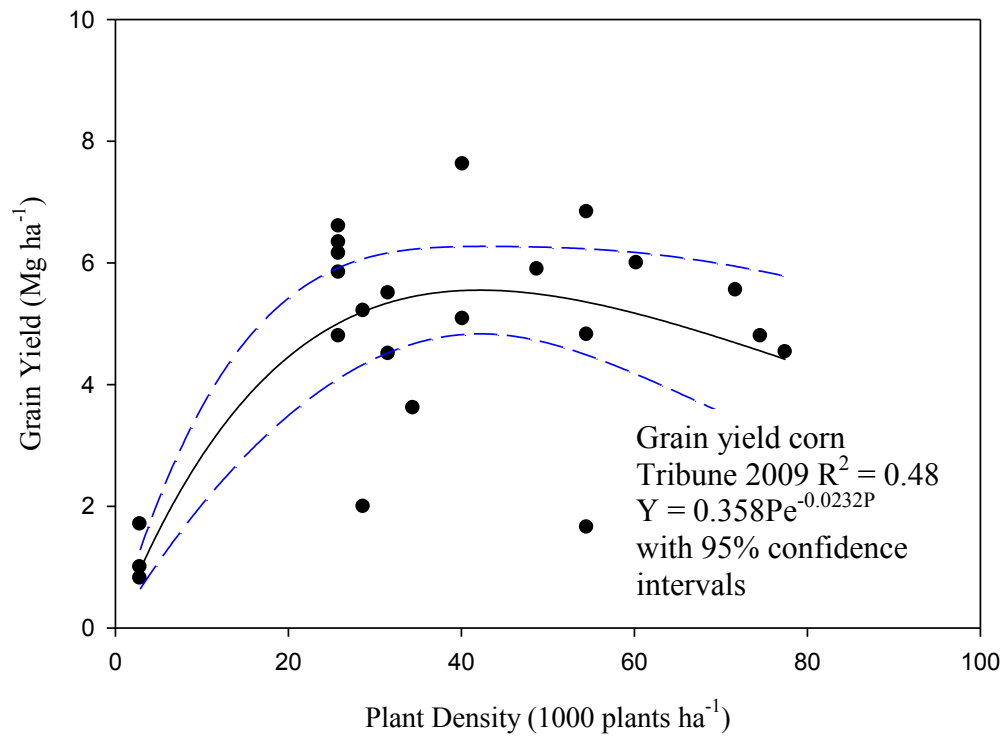


Figure 1.12 Corn grain yield response fit to Duncan yield equation in Tribune, KS in 2009.
Each replication represents a unique emerged population.

Table 1.3 Components of yield for corn and sorghum cellulosic ethanol

Crop	Loc	Year	Field Results			Forage Profile after H ₂ SO ₄			Ethanol Yield		
			HV Mg ha ⁻¹	Stover Mg ha ⁻¹	Total Mg ha ⁻¹	Cell g g ⁻¹ stover	Hemi g g ⁻¹ stover	Lignin g g ⁻¹ stover	Sugar mg g ⁻¹ stover	Ethyl mg g ⁻¹ stover	Ethyl kg ha ⁻¹
Corn	Man	2009	12.6 [*]	6.9 ^{**}	19.5 ^{**}	0.383	0.262	0.162	200 ^{***}	94.0 ^{***}	647
	Trib	2009	10.6 [*]	12.1 ^{**}	22.7 ^{**}	0.385	0.269	0.161	235 ^{***}	90.2 ^{***}	1090
GS	Man	2009	6.93	6.44	13.4	0.521	0.039	0.175	188	77.2	501 ^B
	GC	2009	5.45	2.77	8.2	0.540	0.064	0.113	162	65.0	166 ^{II}
	Man	2010	7.71	7.87	15.6	0.531	0.049	0.125	204	73.8	579 ^a
	Hut	2010	7.65	5.03	12.7	0.534	0.086	0.118	172	62.6	563 ⁱⁱ
SwS	Man	2009	46.9 [*]	22.6	22.6	0.579	0.072	0.147	195	64.4	1460 ^A
	GC	2009	16.8 [*]	14.1	16.8	0.516	0.10	0.103	299	117	1650 ^l
	Man	2010	WD	WD	WD	0.572	0.55	0.150	158	59.8	WD
	Hut	2010	34.9	19.0	19.0	0.574	0.96	0.107	210	75.9	1360 ⁱ
DP	Man	2009	17.0 [*]	16.6	33.6	ND	ND	ND	211	88.7	1440 ^A
	GC	2009	4.5	4.8	9.3	0.539	0.079	0.101	161	79.0	361 ^{II}
	Man	2010	10.8	19.1	29.9	0.547	0.075	0.109	185	66.6	1270 ^a
	Hut	2010	5.13	10.5	15.9	0.579	0.59	0.135	200	72.5	734 ^{i,ii}
PS	Hut	2010	NA	19.4	19.4	0.579	0.075	0.128	205	74.4	1120 ^{i,ii}

*field populations with significant fit to models reflected as highest yielding treatment

**at maximum grain yield

***from Agrupis 2010 using material grown for this paper

Capital letters compare Manhattan, 2009, lower case letters compare Manhattan, 2010, Upper case Roman numerals compare Garden City, 2009, and lower case Roman numerals compare Hutchinson, 2010

Sugar yield g^{-1} , ethanol yield g^{-1} , and Ethanol yield ha^{-1} reflect an assumption of 30% loss in mass during H_2SO_4 hydrolysis (Bansal, 2010)

ND: No data, WD: wind damage, HV: high value yield grain for all except sweet sorghum juice, Loc: location

Corn

Overall biomass and grain yield were curve fit to the Russell biomass model and the Duncan yield model respectively at the $\alpha = 0.05$ level. Variability in both biomass and grain yields were lower at in Manhattan than Tribune in both cases as indicated by the lower coefficients of determination. This is consistent with historical results of these models performing working better in environments with less environmental stress (Duncan, 1984). In Manhattan overall biomass was fit to $M = P (0.03P + 1.78)^{-1}$ (Fig 1.9) with an R^2 of 0.80. The asymptote of biomass is 33.3 Mg ha^{-1} . The asymptote implies that without management adjustments other than population no more than 33.3 Mg ha^{-1} could be produced in this environment with this hybrid. Grain yield in Manhattan was fit to $Y = 0.416Pe^{-0.0120P}$ with an R^2 of 0.81 (Fig. 1.10). Local extremes occur when the first derivative is zero. Since only one local extreme exists in the Duncan grain yield model when $dY dP^{-1} = 0$, P_{\max} has been achieved. In Manhattan $dY dP^{-1} = 0$ when $P = 83,300 \text{ plants ha}^{-1}$. The yield at this population is predicted to be 12.8 Mg ha^{-1} . Tribune biomass was fit to $M = P (0.036P + 0.348)^{-1}$ (Fig. 1.11) with an R^2 value of 0.74. The asymptote in Tribune was 27.8 Mg ha^{-1} . Grain yield in Tribune was fit to $Y = 0.358Pe^{-0.0232P}$ (Fig. 1.12) with an R^2 value of 0.48. In Tribune P_{\max} was $43,100 \text{ plants ha}^{-1}$ with a maximum yield of 5.68 Mg ha^{-1} . Equation three and equation five transformations of figures eight through eleven were reported in Ballard et. al, 2011.

Tribune yield was more sensitive to density than Manhattan. A reduction in density of $10,000 \text{ plants ha}^{-1}$ resulted in a loss of 3.1% of yield or 0.18 Mg ha^{-1} in Tribune. The same reduction in density in Manhattan resulted in a 0.8% loss or 0.10 Mg ha^{-1} . The loss differences were magnified at 20,000 below optimum density. Tribune lost 14.8% or 0.84 Mg at $P_{\max} - 20$. Manhattan lost 3.4% or 0.43 Mg . Over seeding yield losses were not as large as under seeding losses. At $P_{\max} + 10$ Manhattan and Tribune lost 0.7% and 3.4% respectively. At $P_{\max} + 20$ the losses were 3.5% and 8.0%. This was reflected graphically in figures 3 and 4 by smaller derivatives above P_{\max} relative to those below P_{\max} . When changes in density are expressed as a percentage of P_{\max} resulting yield losses are identical at both locations.

Fermentation

Density was not found to have a significant effect on mg ethanol g^{-1} stover in sorghum or corn. Ethanol yields for each variety, location, and year were compared using the highest field

yielding density treatment when linear responses had significant R^2 values. Corn fermentation was reported in Agrupis, (2011).

Final sorghum cellulosic ethanol yields ha^{-1} had a significant year by location interaction. In Manhattan in 2009, sweet sorghum bagasse had the highest ethanol yields with 1460 kg ha^{-1} . In Manhattan in 2010, a significant difference did not exist between the two crops which lived until the end of the year. Dual purpose sorghum's cellulosic ethanol yield of 1270 kg ha^{-1} was different from grain sorghum's ethanol yield of 579 kg ha^{-1} at $\alpha = 0.08$. In 2009 in Garden City, sweet sorghum bagasse produced the most ethanol with 1650 kg ha^{-1} . Garden City bagasse was the only treatment to have over $215 \text{ mg C}_6\text{H}_{12}\text{O}_6 \text{ g stover}$. This higher glucose release represents remaining glucose in the bagasse after juicing. The other sweet sorghum treatments lost that sugar content before drying due to improper refrigeration. The higher sweet sorghum bagasse yield in Garden City came a higher concentration of glucose being left in the stalk after juicing than in Manhattan. In 2010 in Hutchinson, sweet sorghum bagasse produced the most ethanol ha^{-1} with 1360 kg ha^{-1} .

Corn cellulosic ethanol results could not be statistically compared to sorghum results as corn was planted as a separate experiment. Corn cellulosic ethanol yields were significantly different by location. Tribune produced 1090 kg ha^{-1} of ethanol and Manhattan produced 647 kg ha^{-1} of ethanol. Table 2.3 shows the steps toward calculating final ethanol yields.

Discussion

Each crop under consideration has uses other than cellulosic ethanol. Corn and grain sorghum are produced primarily for grain. Dual purpose forage sorghum potentially produces more grain than grain sorghum, but the grain is not accessible with current harvest technology so it is used for forage and silage. Photoperiod sensitive sorghum is used for forage as well.

Starch based ethanol requires less land per unit of ethanol on land capable of producing starch crops. A six Mg ha^{-1} grain yield from corn produces approximately 2.2 Mg ha^{-1} of starch based ethanol (Pimental and Patzak, 2005). The highest yielding treatment within the current study was 1.7 Mg ha^{-1} of cellulose based ethanol. This does not imply cellulosic ethanol will not have a role in reducing indirect land use changes for biofuel production. Excess starch crop residue is available for cellulosic ethanol. In addition land with poor soils or diminishing water

supplies cannot support starch crops. Use of these lands for perennial biomass crops does not displace production of starch crops.

Corn's production of cellulosic material is in excess of what is needed to protect soils from erosion beyond NRCS tolerable values (T). How much corn stover can be removed without allowing erosion levels to exceed Natural Resource Conservation Service (NRCS) guidelines is unclear at this time (Farrell et al., 2006) and will vary according to soil organic matter content, slope length, slope pitch, and soil texture. The proportion of corn stover which can be removed without exceeding NRCS erosion guidelines will vary with location due to soil structure, and climatic influences such as wind and rainfall intensity. Conservation of soil organic matter must also come into consideration. Considerations for removal of grain sorghum biomass are similar to those of corn. Higher sorghum populations create additional water bars (stalk bases) to slow erosion over those water bars of the bases of corn stubble.

Sweet sorghum has alternative yield components to corn and grain sorghum. Sweet sorghum's primary feedstock for ethanol is juice. Juice is also used for sugar and molasses production. A production challenge for sweet sorghum was revealed in this study. The height of mature sweet sorghum provides lodging opportunities both from wind and from the weight of seeds. When breeding for sweet sorghum, improvement in lodging resistance must be considered either through use of a single dwarf gene, thickening of the rind, strengthening the rind through alternative methods, or a combination of these options. Removal of sweet sorghum from the field for cellulosic ethanol production does not present an additional erosion risk since the material is already removed for juice extraction.

In the three year location combinations where sweet sorghum stood until the end of the year, it was the highest cellulosic ethanol yielding crop. The wind event which lodged the sweet sorghum in Manhattan created a large amount of damage in the region including downed trees, and damage to other research plots. Assuming the sweet sorghum plants were four m tall with a 5 cm base at the time of the wind event and the center of wind force occurred half way up the plant, the base of the plant received 19 N m of torque. Only breeding for shorter plants can avoid torque in high wind events.

Dual purpose forage sorghum is the most consistent producer of cellulosic material. Although it does not have the potential to produce as much biomass as sweet sorghum and photoperiod sensitive sorghum, it has better resistance to lodging due to use of a single dwarf

gene. Dual purpose sorghum's high grain production in moist environments cannot be used for ethanol production as a grain only feedstock due to separation challenges. Use of its grain for ethanol would require a multistep process of supplying amylase to a grain/biomass mix, fermenting the starch sourced sugars, distilling the first ethanol product, and then commencing a cellulosic ethanol process.

Use of photoperiod sensitive sorghum for ethanol provides an option of the cellulosic route only. Availability of photoperiod sensitive sorghum feedstock for ethanol will occur primarily when hay prices are low, which gives agricultural producers incentive to search for alternative markets. One advantage of photoperiod sensitive sorghum for cellulosic feedstock is micronutrients cannot be transported to grain. This provides the potential for a reduced micronutrient supply cost at bio-processing facilities.

Conclusions

Response to density was more reliable in corn than sorghum. The coefficients of variation were significant in all four corn models. Manhattan had a better fit to both the corn grain model and the corn biomass model. Use of the Duncan grain yield model on farm will reveal an ideal population unique to field conditions and hybrid. Corn biomass models are most useful when biomass values justify a shift in harvest index. Sorghum density models did not consistently demonstrate significant patterns. This result is consistent with other research with sorghum's relative management insensitivity.

Sweet sorghum cellulosic ethanol yields were the highest of any of the crops studied when the plant remained standing until the end of the growing season, but lodging must be addressed to ensure consistent product availability.

Dual purpose forage sorghum produced ethanol better than grain sorghum in Manhattan, but not in dryer environments. Dual purpose forage sorghum's grain production was higher than grain sorghum as well, but the grain yield cannot be utilized due to the constraints of current harvest technology. Dual purpose sorghum did not experience lodging in 2010 as its shorter height avoided some of the torque created by wind. Yields in dry environments were not different from grain sorghum. Grain sorghum is better suited in a dry environment as a grain crop than as a cellulosic ethanol feedstock.

A poor harvest index in dry environments leads to better cellulosic ethanol potential than corn in Manhattan. Removal of cellulosic material from land in western KS presents a risk of wind erosion. Use of stover for cellulosic material should be limited by erosion considerations.

Milligram of ethanol g^{-1} of biomass was not affected by population in sorghum and by 4% in corn. Changes to this ratio have alternative routes for additional research. Breeding for more leafy plants could also decrease overall lignin concentration.

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Chapter 2 - Annual Oil Yield Totals from Crop Combinations in KS for Biodiesel Feedstocks

Abstract

Oilseed crops have tremendous potential for conversion to biofuels due to direct conversion of vegetable oils to biodiesel. Eleven crop combinations were planted in Manhattan and Hutchinson, KS in 2010 and 2011. Evaluate crops or combination of crops which maximize oil per year and to produce oils for lipid profiling. Each treatment could be grown in the year following a summer annual, thus eliminating winter crops. Full season soybeans were consistently the highest oil yielding single crop. At Manhattan in 2010, maturity group IV soybean yielded the highest annual oil total. At Hutchinson in 2010 maturity IV and V soybean produced the most oil. Soybean had the highest net energy productivity due to the fuel input necessary for a second planting and harvest. In 2011, full season soybean maturity group V produced the most oil in both locations. Full season soybean was the most reliable producer of biodiesel feedstock oil among the crops considered. Double crop options were shown to not be viable with the exception of sesame.

Introduction

Biodiesel production is an essential component to fulfilling the goals of the Energy Independence and Security Act (U.S. House, 2007). Soybean oil is the primary feedstock for biodiesel in the United States (Stroup, 2004). Soybean oil is projected to remain the largest supplier of biodiesel feedstocks with a 47% market share in the 2012 and 2013 marketing years (Weber, 2011) and increased demand for biodiesel will require diversification of feedstocks. Intensification of cropping systems may decrease the demands that biofuels have created on grain supplies production of oilseeds whose meals can be used for feed and food. This approach will also limit the effect of biofuels on grain supplies.

Soybean, sesame, canola, camelina, safflower, and sunflower can all be used to produce oil for biofuels and meal. All of the meals from these crops have been FDA approved for human or animal consumption. Sesame and soybean are approved for protein supplements. Camelina and canola are approved for swine (*Sus scrofa* L.) feed, (Galasso et. al, 2011; Carter et. al. 2004).

Sunflower and safflower meals are approved for dairy cattle (*Bos Taurus* Bojanus) feed (Hale et al., 1991). The oils can also be used for biodiesel as yellow grease after being used in the food industry.

Market sizes, distribution of uses, and values of each oil vary between species. May 2012 soybean futures have been valued between \$1.11 kg⁻¹ and \$1.27 kg⁻¹ since January of 2012 (CBOT, 2012). June sunflower oil futures were valued at \$7.57 kg⁻¹ (Commodity Online, 2012). Sesame and safflower oils are not traded in large volume in the United States. Both are available for purchase in bulk online (alibaba.com). Sesame oil was available for \$5.90 kg⁻¹ and safflower oil was available for a wide range prices from \$1 kg⁻¹ to \$20 kg⁻¹.

Polyunsaturated fats are desirable for health supplements and chemical engineering applications. The lipid profile of each crop determines the crops desirability for these applications. Brassica seeds have a more desirable lipid profile in this aspect than legumes (Vollman et al, 2011, Upchurch et. al, 2010)

Economic demand for biodiesel will directly increase demand for oil seeds creating an incentive for investment in the improvement of oil yields (Hanna et. al. 2005). National mean canola yields improved from 1470 to 2000 kg ha⁻¹ from 2001 to 2011 (NASS). Camelina production has attracted attention to provide an alternative to continuous oat production in Montana, North, and South Dakota. Camelina is being considered in areas that no longer have adequate water supplies for irrigation to produce corn in California. When winter canola is produced in Kentucky and Tennessee harvest occurs in time to grow a full season double crop soybean (Murdock et. al. 1992). Safflower can be grown on land that receives between 40 and 130 cm of rainfall year⁻¹ (Tuck et al, 2005).

For our study, oil crops were selected that could be used in a cellulose-oil crop rotation. Spring canola and spring camelina were selected for the cool season treatments as they can be planted following a sorghum (*Sorghum bicolor* L.) crop. Planting winter canola or camelina after sorghum in KS does not allow adequate time for stand establishment prior to the onset of winter and often leads to severe yield losses.

Common pathogens between oil seed crops limit the potential for following soybeans with mustard crops and vice versa without scouting for fungal infections. *Sclerotinia sclerotiorum* (Lib) spores are present in soybean and canola fields (Lu, 2003). Sunflower is also susceptible to *Sclerotinia spp.* High spore counts present a risk of crop failure when these crops

are planted continuously. Reduction of this risk with the application of a fungicide adds expense to production. Sesame is not susceptible to *Sclerotinia spp* and could prove useful in breaking this disease cycle.

Oilseed yields and oil concentration are influenced by several factors. Oil concentration is a quantitative genetic trait in soybean (Panthee et. al. 2005). Late seeding times and excessive N fertilization have been reported to decrease oil concentration in canola (Hocking, 2001). Camelina oil concentration has been reported to be inversely proportional to seed size (Vollman et al, 2007). Oleic acid (18:1 cis-9) concentrations decreased in safflower oil when high oleic acid cultivars were grown in saline soils (Irving et. al. 1988). This change makes safflower oil less desirable for soap and pharmaceutical applications. Sunflower maturity has been a closely linked trait to seed oil concentration (Leon et al., 2003). The range of environments in Kansas creates an opportunity to review the effects of temperature and rainfall differences in oil production in each of these crops

Water is often the limiting resource in the Great Plains. Double cropping creates even more limited soil water availability for the second crop. Once a stand is established, the timing of drought stress does not significantly affect canola yield, but the intensity of the stress does affect yield (Nielson, 1997). Sionit and Kramer (1977) found that soybean is most susceptible to osmotic stress during the pod set and early filling stages. They also noted no changes in oil concentration from water stress at flower induction, flowering, pod formation, or pod filling. A 20% cumulative reduction in seasonal evapotranspiration resulted in a 24% decrease in camelina yield in AZ (French et al., 2009). Double crop systems are most common and successful in regions within 40° of the equator which receive over 100 cm of rain per year. Areas more than 40° north or south do not have adequate growing season length to support a second annual crop. Areas with less than 100 cm of expected annual precipitation often have less than 10 cm of equivalent water depth in the first 150 cm of soil after the first crop of a year is harvested. This low soil water content makes stand establishment of a second crop difficult.

Intensification of oil seed production in KS may provide an increase in biodiesel feedstock availability. As a result we had two objectives. 1.) Determine the oil production of eleven individual crops and combinations. 2.) Determined if soybean, sunflower, safflower or sesame double crops were reliable double crop options behind spring camelina and spring canola.

Materials and Methods

A randomized complete block design with four replications was used in Manhattan, KS and Hutchinson, KS in 2010 and 2011. In Manhattan, the soil type was a Rossville silt loam (fine-silty, mixed, superactive, mesic, Cumulic Hapludoll). In Hutchinson, the soil type was a Funmar-Taver loam (fine-loamy, mixed, superactive, mmesic Pachic Argiustolls – fine, smectic, mesic, Udertic argiustolls) in 2011. In 2010, the soil type in Hutchinson was a Naron sandy loam (fine-loamy, mixed, superactive, mesic Udic Argiustolls).

To initiate field studies spring canola ‘1651h’ (Clearfield Croplan Genetics St. Paul, MN) and camelina ‘Cheyenne’, (Blue Sun Biodiesel Golden, CO) were planted using a Truax seed drill (Truax New Hope, MN) on 1 March in 2010 and 11 March in 2011 in Manhattan and the following day in Hutchinson. Nitrogen was applied by broadcasting urea at a rate of 67.3 kg N ha⁻¹ 15 to 20 days after planting. Soil test results indicated a sulfur deficiency for Manhattan in 2011. Gypsum was applied pre-emergence at a rate of 22 kg S ha⁻¹. Weed control was accomplished with a pre-plant burn down application of Roundup Weathermax at a rate of 2.3 liter ha⁻¹ (1.5 kg a.i. glyphosate ha⁻¹) and Prowl at a rate of 1.2 liter ha⁻¹ (0.53 kg ha⁻¹). A pre-emergence application of Ignite at a rate 2.3 liter ha⁻¹ (0.47 kg a.i. glufusinate-ammonium) and Poast Plus at a rate of 1.8 liters *ha⁻¹ (0.32 kg a.i. ha⁻¹ sethoxydim) was also given. No further herbicides were applied to the spring crops in 2010.

In 2011, stand establishment was poor due to a post plant drought. A post emergence application of Raptor at a of 0.29 liter ha⁻¹ (0.035 kg ha⁻¹ a.i. ammonuim salt of imazamox) was applied on the canola to rescue the stand on 9 May in Manhattan. No post emergence broadleaf herbicide could be applied to the camelina. Both canola and camelina received a post emergence application of sethoxydim at the same rate as the pre emergence treatment to control grassy weeds.

Spring crop harvest occurred at physiological maturity. Camelina matured more quickly than canola in both years. In 2010 harvest was completed by harvesting 6.97 m² samples using hedge shears. Due to concerns of shattering when bagging the 2010 samples, spring crop harvest in 2011 was completed using a Gleaner E III plot combine (AGCO, Duluth, GA). Harvested areas were 18.1 m² samples. Two grain samples were taken from each replication for the spring crops. Camelina harvest occurred on 21 June in 2010 and 29 June in 2011 in Manhattan. Canola harvest occurred on 2 July in 2010 and 9 July in 2011 in Manhattan. Hutchinson harvest occurred 22

June, 2010. Camelina failed in Hutchinson in 2011 due to a severe drought and only vegetative biomass was produced by canola in Hutchinson. The canola biomass in Hutchinson was harvested on 13 July.

Double crop planting occurred shortly after spring crop harvest. Sesame 'S-33' (Sesaco Paris, TX) and safflower 'CL99' (Calwest Seeds Woodland, CA) were planted with the same drill as the spring crops. Sesame was planted at a rate of 7.9 kg ha⁻¹. Safflower was planted at a rate of 33 kg ha⁻¹. Soybean 'KS 3406' (KS St Univ. Foundation seed Manhattan, KS) and sunflowers '3080 DMR, NA' (Croplan Genetics St. Paul, MN) were planted using a John Deere 7200 (John Deere, Moline, IL) planter modified into a two row plot planter. Soybeans were planted at a rate of 300,000 seeds ha⁻¹. Sunflowers were planted at a rate of 69,000 seeds ha⁻¹. In 2010 double crop plantings occurred the same day as spring crop harvest. In 2011 crops after camelina were planted on 6 July in Manhattan. Crops after canola were planted on 11 July in Manhattan. Pre emergence glyphosate was applied at a rate of 2.3 a.i ha⁻¹ in 2010 to all double crops both years. On 24 June the plots with camelina treatments in Hutchinson were sprayed with 2-4,D at a rate of 1.2 liter ha⁻¹ (0.84 a.i. kg ha⁻¹ 2,4-dichlorophenoxyacetic acid) to kill emerged weeds prior to double crop planting. A residual weed control treatment was added pre emergence in 2011 due to palmer amaranth (*Amaranthus palmeri* S. Wats.) infestation in 2010. Double crops were planted on 7 July, 2011 in Hutchinson. Double crops after camelina in Manhattan were planted on 29 June, 2011. Crops after canola in Manhattan were planted on 11 July, 2011. Residual herbicide applications were dual II Magnum at a rate of 1.2 liters ha⁻¹ (0.92 kg a.i. ha⁻¹ S-metolachlor) for sesame and safflower for both locations. Spartan Charge at a rate of 0.62 liters ha⁻¹ (0.26 kg ha⁻¹) was the residual herbicide application for sunflower and soybean in both locations. Nitrogen was applied to sunflowers at a rate of 112 kg N ha⁻¹ using urea. Sesame received 67.3 kg N ha⁻¹ as urea.

Sesame in Manhattan was the only successful double crop in 2010. No double crops were successful in 2011. Sesame samples were harvested from 6.97 m² area after the first night below 0° C for more the four hours.

Full season crops were maturity group IV soybeans 'KS 4702' in 2011 'KS 4610' in 2010 (KS St. Univ. Manhattan, KS Foundation Seed), maturity group V soybeans 'KS 5507' (KS St. Univ. Foundation Seed), and '559 CL, DMR, NS' (Croplan Genetics St. Paul, MN) sunflowers. They were planted with the same planter as the soybeans and sunflowers used as

double crop treatments and at the same rate. Plantings occurred on 15 and 16 May in 2010 in Manhattan and Hutchinson, respectively. In 2011, the full season planting occurred on 17 and 18 May, at Manhattan and Hutchinson respectively. Nitrogen was applied 15 to 20 days after planting using urea at a rate of 112 kg ha⁻¹ to the sunflowers. Sunflowers were treated with Warrior insecticide at a rate of 0.28 liters ha⁻¹ (140 g a.i. lambda-cyhalothrin ha⁻¹) at the R3 stage both years.

Full season crops were harvested at physiological maturity using 3.48 m² samples. Soybeans were harvested using hedge shears. Sunflower heads were harvested using rose clippers. Remaining sunflower biomass was harvested using a machete. All seeds were cleaned using a stationary thresher.

Total oil year⁻¹ ha⁻¹ required extraction of oil from seed subsamples. The Kansas State University Animal Science Analytical Laboratory used AOAC method 920.39 to extract oil using a Goldfitch fat extractor. Oil concentration was reported on a dry seed basis. Moisture concentrations were determined by comparing mass of laboratory delivered samples to oven dried samples. Oil totals for each crop and annual totals were compared using the F test analysis of variance. The PROC-MIX function of SAS 9.3.1 was used to determine the least significant difference.

Results were compared in analysis of variance using Tukey's adjustment for multiple mean comparison in SAS version 9.3 PROC GLM.

RESULTS AND DISCUSSION

Growing conditions varied by year. In 2010, Manhattan experienced conditions near the 30 year average for temperature and precipitation (Table 3.1). The conditions at Hutchinson were drier than the 30 year average in the spring and wetter than the 30 year average in the summer of 2010 (Table 3.2). An extreme drought occurred in Hutchinson in 2011 with Manhattan receiving a less extreme drought (Table 2.1 and 2.2). A high wind event of 145 km hour⁻¹ resulted in the loss of sunflowers in 2010. Stand establishment was hampered by drought for all crops in 2011. Maturity group IV soybean was replanted 14 days after initial planting in both locations due to poor establishment in 2011. Maturity V soybean and full season sunflower did not require a 2011 replanting. Sesame, double crop sunflower and double crop soybean emerged both years in Manhattan. In 2010, double crop soybean achieved a height of less than 15 cm and had fewer

than four pods per plant. Double crop soybean was not harvested as these yields are below an economic value of the harvest cost. In 2011, double crop soybean height was close to 2010 and fewer than 30,000 plants ha⁻¹ emerged. In Manhattan, sesame was killed by fusarium wilt in 2011. Double crop sunflower died prior to R1 in Manhattan in 2010 for unknown reasons. In 2011, Manhattan double crop sunflower emerged and was eaten by deer within 10 days. In 2010 at Hutchinson, sesame was the only double crop treatment to emerge.

Year, location, and treatment were all significant at $\alpha = 0.05$ for all oil yield variables. Manhattan in 2010, oil yields were the highest compared with any year, location combination. In 2010 Manhattan treatments collectively yielded 3.9 times more oil as Hutchinson. In 2011 only maturity V soybean produced reproductive mass in Hutchinson. In Manhattan in 2011, all of the first crops planted in each treatment produced dry matter. The first crop seed dry matter in Manhattan in 2010 was 1.86 times the 2011 yield. No second crop dry matter was produced in 2011 in either location. Sesame was the only second crop to have seed yield in 2010 (Table 3.3).

Full season soybean was the most reliable crop. Maturity IV soybean in Manhattan produced the highest yields in 2010 of any year with 3240 kg ha⁻¹. Maturity IV soybean yields (1250 kg ha⁻¹) were less than maturity V soybean in 2011 in Manhattan. Poor stand establishment hampered early season growth for the maturity IV soybeans in 2011 in Manhattan. Maturity V soybean produced the most seed dry matter in 2011 in Manhattan (1840 kg ha⁻¹) and was the only seed bearing crop in Hutchinson in 2011 (157 kg ha⁻¹). Soybean oil concentrations were not different between locations, years, and varieties (Table 3.3).

Spring crop seed yields varied between location and year. Camelina was the highest seed yielding crop in Manhattan in 2010 with 999 kg ha⁻¹. The other spring crop treatment and year combinations were not different for seed yields. Canola and camelina oil concentrations were both significantly less in 2011 than 2010.

Full season sunflower produced harvestable yield only in Manhattan in 2011. In Hutchinson in 2011, heads were formed, but no seeds were produced. In 2010, a high wind event broke the stalks prior to the onset of seed in Manhattan. Avian pests removed all yield in 2010 at Hutchinson.

Sesame was the only double crop to produce seed in both locations in 2010. No double crops produced seed in 2011. In both locations, sesame produced more seed in 2010 after camelina than after canola. Yields in Manhattan were higher than yields at Hutchinson after both

camelina and after canola. Oil concentrations ranged from 55.3% on a mass basis in Manhattan after camelina to 50.8% in Hutchinson after canola.

Oil yields were affected by both location and year as indicated by the significant interaction of the two. Thus oil yields were compared within each year and location. In Manhattan in 2010, maturity IV soybean produced the most oil at 642 kg ha⁻¹ (Table 2.3). The second most oil for individual crops in Manhattan in 2010 were camelina at 365 kg ha⁻¹ and maturity V soybean with 373 kg ha⁻¹. In Hutchinson in 2010, no significant difference existed between oil yields. In 2011 in Manhattan, maturity V soybean produced the most oil with 354 kg ha⁻¹. Maturity IV soybean and sunflower produced the second most oil in Manhattan in 2010.

Total oil yield varied with year, crop, and location. In 2010 maturity IV soybean produced the most oil in Manhattan at 642 kg ha⁻¹. Camelina followed by sesame produced the second most oil at 546 kg ha⁻¹. In Hutchinson in 2010, camelina followed by sesame, canola followed by sesame, canola without a second crop and both full season soybeans produced the most oil. In 2011, maturity five soybean yielded the most oil in Manhattan and was the only crop to produce seed in Hutchinson. Table 2.3 summarizes the yield calculations for oil.

Discussion

Production of oilseeds other than full season soybean presented several challenges. Sunflower pest management requires an extra field pass during early reproductive stages. This adds additional cost from labor, equipment use, and insecticide purchases. Even with these incurred costs, only one out of four full season sunflower year and location combinations produced a viable crop and the successful sunflower crop produced less than soybean. Camelina demonstrated potential in 2010 in Manhattan. The 2011 camelina yield in Manhattan reflects the need for a rainfall event shortly after planting to establish camelina. Rotating camelina in a field with sprinkler irrigation to provide establishment water and conserve water the rest of the season appears to be a more reliable production option for camelina. Canola yields in Manhattan were about one tenth of the national average in both years. This result demonstrates canola should be produced as a winter crop in KS, not as a spring crop.

Full season crops appeared to have the most promise. Equal oil production between the sum of two crops and full season soybean implies soybean is the better choice. Fuel use and other expenses are more for double crop systems due to additional field passes are nutrient

replacement needs. Full season sunflowers may also have improved yields with further pest management. A ring of sunflowers surrounding experimental plots will serve as a deterrent of birds to attract them to the sacrificial ring rather than the plots.

Limited results exist with sesame and camelina production in KS. Full season sesame may produce more oil than full season soybean with the water, heat, and time assets of a full summer.

Table 2.1 Monthly temperature and rainfall summary for Manhattan.

Month	Monthly average air temperature			Monthly precipitation totals		
	2010	2011	30 yr avg	2010	2011	30yr avg
	-----°C-----			-----mm-----		
March	7.10	6.85	6.44	44.7	33.8	65.8
April	15.2	13.4	12.5	58.7	63.2	78.0
May	17.4	18	17.8	92.0	131	91.4
June	25.3	25.2	22.8	168	121	168
July	27.1	29.7	26.1	106	52.8	107
August	26.9	27.3	25.6	81.3	59.2	88.9
September	21.8	19.2	21.0	76.2	37.1	68.6

Source: Kansas St. Univ. Weather Data Library

Table 2.2 Monthly temperature and rainfall summary for Hutchinson 2011

Month	Monthly average air temperature			Monthly precipitation totals		
	2010	2011	30 yr avg	2010	2011	30yr avg
	-----°C-----			-----mm-----		
March	5.5	4.47	6.94	35.8	8.63	54.6
April	10.8	11.8	11.9	57.9	37.8	63.3
May	17.7	14.6	17.8	122	46.5	96.5
June	26.2	22.6	26.1	199	38.9	109
July	27.2	26.6	27.2	155	120	88.9
August	26.9	25.1	26.7	100	67.8	78.7
September	21.9	16.9	22.2	32.8	7.62	83.8

Source: Kansas St. Univ. Weather library

Table 2.3 Seed Dry Matter, Oil Concentrations, and Oil Yields Compared within Location and Year using Tukey's Test at $\alpha = 0.05$

Crop	Location	Year	Crop 1 Seed Dry Matter kg ha ⁻¹	Crop 2 Seed Dry Matter kg ha ⁻¹	Crop 1 Oil Concentration 100(g oil g ⁻¹ dry matter)	Crop 2 Oil Concentration 100(g oil g ⁻¹ dry matter)	Crop 1 Oil kg ha ⁻¹	Crop 2 Oil kg ha ⁻¹	Total Annual Oil kg ha ⁻¹
Camelina and sesame	Manhattan	2010	999 ^C	365 ^A	35 ^{AB}	55 ^A	345 ^B	201 ^A	546 ^B
	Hutchinson	2010	81 ^c	193 ^B	31 ^{ab}	52 ^C	25 ^a	100 ^B	125 ^a
	Manhattan	2011	175 ^{III}	0	23 ^{I,II}	0	40 ^{III}	0	40 ^{III}
	Hutchinson	2011	0	0	0	0	0	0	0
Camelina and safflower, sunflower, or soybean	Manhattan	2010	999 ^C	0	34.7 ^{AB}	0	345 ^B	0	345 ^C
	Hutchinson	2010	81 ^c	0	31 ^{ab}	0	25 ^a	0	25 ^a
	Manhattan	2011	175 ^{III}	0	22 ^{I,II}	0	40 ^{III}	0	40 ^{III}
	Hutchinson	2011	0	0	0	0	0	0	0
Canola and sesame	Manhattan	2010	171 ^D	303 ^A	40 ^A	54 ^B	68 ^C	164 ^A	231 ^E
	Hutchinson	2010	247 ^{bc}	41 ^C	41 ^a	51 ^D	102 ^a	21 ^C	123 ^a
	Manhattan	2011	127 ^{III}	0	21 ^{I,II}	0	27 ^{III}	0	27 ^{III}
	Hutchinson	2011	0	0	0	0	0	0	0
Canola and safflower, sesame, or sunflower	Manhattan	2010	171 ^D	0	40 ^A	0	68 ^C	0	68 ^F
	Hutchinson	2010	247 ^{bc}	0	41 ^a	0	102 ^a	0	102 ^a
	Manhattan	2011	127 ^{III}	0	21 ^{I,II}	0	27 ^{III}	0	27 ^{III}
	Hutchinson	2011	0	0	0	0	0	0	0
Maturity 4 soybean	Manhattan	2010	3240 ^A	NA	20 ^B	NA	642 ^A	NA	642 ^A
	Hutchinson	2010	762 ^a	NA	16 ^b	NA	123 ^a	NA	122 ^a
	Manhattan	2011	1250 ^{II}	NA	20 ^{II}	NA	234 ^{II}	NA	234 ^{II}
	Hutchinson	2011	0	NA	0	NA	0	NA	0
Maturity 5 soybean	Manhattan	2010	1910 ^B	NA	20 ^B	NA	373 ^B	NA	373 ^D
	Hutchinson	2010	701 ^{ab}	NA	17 ^b	NA	121 ^a	NA	122 ^a
	Manhattan	2011	1840 ^I	NA	20 ^{II}	NA	354 ^I	NA	354 ^I

	Hutchinson	2011	157	NA	20 ^b	NA	32	NA	32 ^e
Sunflower	Manhattan	2010	0	NA	0	NA	0	NA	0
	Hutchinson	2010	0	NA	0	NA	0	NA	0
	Manhattan	2011	547 ^{III}	NA	39 ^I	NA	229 ^{II}	NA	229 ^{II}
	Hutchinson	2011	0	NA	0	NA	0	NA	0
Standard error			83.7	16.6	3.22	0.20	20.9	8.47	21.1

Capital letters compare Manhattan 2010, lower case letters compare Hutchinson 2010, Roman numerals compare Manhattan 2011 for total oil and first crop oil, first crop dry matter, second crop and dry matter oils are compared by A,B, and C only

CONCLUSIONS

Total oil yield was highest from full season soybean in all locations and all years. Camelina showed promise as an oil crop for the region. The crop combination with the second most oil yield in Manhattan in 2010 was camelina followed by sesame. Quick stand establishment is crucial for the success of camelina due to limited herbicide options. If planted as a winter crop camelina could be harvested earlier allowing for earlier harvest and an extended growing season for sesame.

The other treatments require alternative production methods for success. Canola should be grown as a winter crop in Kansas. Sunflower plots are difficult to maintain without buffer sunflowers surrounding the plot to attract birds away from the experiment. Safflower and double crop sunflower did not survive to physiological maturity in any year, location combination.

Double crop production systems require additional inputs and do not yield as well as full season soybean. Tested double crop systems were not viable under the tested conditions as only one second crop in one location in a single year produced harvestable yield.

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Chapter 3 - Lipid Profiles of Drought Stressed Oil Crops Grown in Kansas

Abstract

Lipid profiles of vegetable oils present an opportunity to separate high value lipids from bulk production. Identifying profiles of agricultural crops allows engineers to identify lipids with double bonds at an end of the C chain for increased reaction kinetics or Ω -3 oils for health supplements. Oil seed crops that included maturity group IV and V soybean (*Glycine max*(L) Merr), spring canola (*Brassica napus* L), spring camelina (*Camelina sativa* L.), and sunflower (*Helianthus annuus* L.) grown in Kansas were analyzed for oil concentration and their lipid profile. All crops were planted in Manhattan and Hutchinson, KS. Oils were extracted using chloroform (CHCl_3) and methanol (CH_3OH) solvent mixture. Extracted oils were profiled for lipid concentration using gas chromatography. All crops produced seed yield in Manhattan and only maturity group V soybean produced seed in Hutchinson in 2011. Oil profiles varied between species and camelina had the most desirable combination of concentrations for the five lipids of most interest.

Material and Methods

A randomized complete block with four replications was used to plant maturity 4.7 ('KS 4702' Kansas St. Univ. Manhattan, KS) and 5.5 ('KS 5507' Kansas St. Univ. Manhattan, KS) soybean (*Glycine max*(L) Merr), canola '1651h Clearfield' (Croplan Genetics St. Paul, MN) (*Brassica napus* L), camelina (Cheyenne, Blue Sun Biodiesel Golden, CO) (*Camelina sativa* L), and sunflowers '559 CL, DMR, NS' (Croplan Genetics St. Paul, MN) (*Helianthus annuus* L.), in Hutchinson, KS and Manhattan, KS in 2011. Canola and camelina were spring crops. Two varieties of soybeans and a 95 day relative maturity sunflower were planted as full season crops. Double crops following the spring crops were soybean, and ninety day relative maturity sunflowers. Drought lead to the failure of all double crops in Manhattan, KS and all crops except maturity five soybeans in Hutchinson, KS. The non legume crops received $112 \text{ kg} \cdot \text{ha}^{-1}$ of (N) from urea ($(\text{NH}_2)_2\text{CO}$) 15 - 20 days after planting. The brassica crops received $22.4 \text{ kg} \cdot \text{ha}^{-1}$ of sulfur (S) from gypsum ($\text{CaSO}_4(\text{H}_2\text{O})_2$) at the same time as the N broadcast application.

Following harvest all crops were dried to 3% moisture prior to oil extraction. Chapter two reviews the crop yields and production methodology.

Five 100 mg seed subsamples from each replication were used for oil separation. The seeds were heated at 75°C for 15 minutes in isopropanol with 0.01 (wt%) BHT. The seeds were then crushed and 1.0 mL chloroform followed by 1.0 mL methanol and 0.8 mL of water were added to separate the lipids from the protein. The mixture was then shaken for 30 seconds and centrifuged for 10 minutes at 10,000 rpm to separate the phases. The supernatant was saved. The extraction was repeated three times adding 1.0 mL of chloroform each time. After extraction, 0.5 mL of 1M KCl was added and the mixture was shaken and centrifuged. The supernatant composed of water was removed and discarded. To remove any proteins remaining, 1.0 mL of water was added and the mixture was shaken and centrifuged. The top layer was again removed and discarded. Samples were then dried under N and the oil weighed using a precision balance (Mettler Toledo AX26 Greifensee, Switzerland). Samples were dissolved in 1000 μ L of chloroform and 25 μ L of this mixture was analyzed using gas-chromatography (GC).

The internal standard used for the analysis was pentadecanoic acid (15:0). The sample and 50 μ L of internal standard (concentration: 1nmol 1 μ L⁻¹) were mixed into screw-cap tube. The solvent was evaporated and 1 mL of 3M methanolic hydrochloric acid was added to each tube and bubbled with N. The tubes were heated at 78°C for 30 min, after which 2 mL of water was added followed by 2 mL of hexane:chloroform (4:1, v/v). The tubes were shaken for 30 sec and then centrifuged for 2 minutes to separate the phases. The upper (hexane:chloroform) layer was separated to a clean tube. Then 2 mL of hexane:chloroform was added to the aqueous phase, shaken and centrifuged. The supernatant was removed and combined with previously extracted organic layer. This extraction was repeated three times. The organic layer was dried under N. The sample was then dissolved in 100 μ L of hexane and transferred to GC vials. The gas chromatography-FID (Flame Ionization Detector) analysis was performed at the Kansas Lipidomics Research Center with a 6890N GC (Agilent Technologies) coupled to a flame ionization detector. The GC was fitted with a HP-88 capillary column with a bis (Cyanopropyl) Polysiloxanes stationary phase (column length: 100 m, internal diameter: 250 μ m, film thickness: 0.25 μ m). Helium was used as the carrier gas at a flow rate of 1.2 mL min⁻¹. The back inlet was operating at a pressure and temperature of 223 kPa and 275°C, respectively. An Agilent 7683 autosampler was used to inject 1 μ L of the sample in the split mode with a split ratio of 10:1. The

GC temperature ramp was operated as follows, initial temperature of 70 °C, ramp 1 at 15 °C min⁻¹ to 175°C, ramp 2 at 1 °C min⁻¹ to a final temperature of 235°C. The flame ionization detector was operated at 260°C. The hydrogen flow to the detector was 30 mL min⁻¹ and air flow was 400 mL min⁻¹. The sampling rate of the FID was 20 Hz. The data were processed using Chemstation software. Lipids that could be used for value added products were sought.

Due to the unbalanced design and large differences in the oils of interest confidence interval were expressed for each lipid instead of using a randomized complete block analysis of variance.

Results

Of the 18 lipids profiled five were chosen for comparison of concentration due to their potential for use in value added products. The standard solution provided by Kansas St. Univ. Lipodomics Research center contained 18 lipids. Five of the lipids in the solution were of interest for value added products. Palmitic acid (C16:0) had the highest concentration in group IV soybean at 4279 nmol μL^{-1} (Table 3.1). The other crops all had approximately 2000 nmol μL^{-1} of C16:0 and were not different. Sunflower had the highest concentration of C18:1(oleic acid) at 48600 nmol μL^{-1} . Canola had the second most C18:1 at 24400 nmol μL^{-1} (Table 3.2). There were no differences between the soybean varieties in Manhattan. Difference in environment appeared to affect the C18:1 concentration between group V soybean from Manhattan and Hutchinson. In Manhattan, 7187 nmol μL^{-1} were reported and Hutchinson had 8038 nmol μL^{-1} . Camelina produced the most C20:1 (arachidonic acid) at 9230 nmol μL^{-1} . Canola produced 1050 nmol μL^{-1} while sunflower produced 375 nmol μL^{-1} of C20:1. The remaining crops had concentrations of 100 nmol μL^{-1} of C20:1. Linoleic acid (C18:2) was present at the highest concentration in the soybean crops, each being around 40000 nmol μL^{-1} . Canola, camelina and sunflower had similar concentrations of C18:2 around 19000 nmol μL^{-1} . Linolenic acid (C18:3) (linolenic acid) offers the greatest opportunity for reactions because of the three double bonds. Camelina's concentration of C18:3 was the highest at 21300 nmol μL^{-1} (table 3.3). Canola and soybean concentrations of 18:3 were near 6000 nmol μL^{-1} . Sunflower had a lower concentration. Table 3.1 summarizes the observed concentrations of saturated fatty acids. Table 3.2 summarizes monounsaturated fats. Table 3.3 shows the concentration of polyunsaturated fats.

Table 3.1 Saturated fatty acids concentration for six crops grown in two locations in KS.

Crop and Location	C14	C16	C17	C18	C21	C24
	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹
Canola Manhattan	55 ±8	1860 ±273	141 +/-19	980 +/-139	593 ±115	139 ±26
Camelina Manhattan	127 ±52	2890 ±1490	-	912 ±181	-	97 ±38
Sunflower Manhattan	96 ±29	2357 ±273	-	1516 ±301	-	257 ±40
Group IV Soybean Manhattan	90 ±51	4280 ±1130	136 +/-58	2040 ±732	94 ±10	116 ±107
Group V Soybean Manhattan	43 ±17	2480 ±575	79 +/-10	-	58 ±7	-
Group V Soybean Hutchinson	41 ±7	2710 ±771	74 +/-14	-	67 ±16	-

Values after each mean represent a 95% confidence interval

Table 3.2 Monounsaturated fatty acids concentration for six crops grown in two locations in KS.

Crop and Location	C16	C17	C18	C20	C24
	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹
Canola Manhattan	377 ±54	208 ±29	24400 ±5950	1050 ±145	243 ±196
Camelina Manhattan	155 ±29	-	6112 ±1192	9230 ±1720	463 ±76
Sunflower Manhattan	277 ±111	-	48600 ±14900	375 ±128	-
Group IV Soybean Manhattan	92 ±15	91 ±32	12700 ±1100	171 ±21	-
Group V Soybean Manhattan	72 ±22	-	7190 ±1570	137 ±26	-
Group V Soybean Hutchinson	81 ±14	-	8040 ±2510	127 ±35	-

Values after each mean represent a 95% confidence interval

Table 3.3 Polyunsaturated fatty acids concentration for six crops grown in two locations in KS.

Crop and Location	C18:2	C18:3	C18:3n6 or C20:0	C20:2	C22:0 or C20:3n6	C20:3n3	C20:4n6	C22:2
	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹	nmol uL⁻¹
Canola	19200 ±3980	5560 ±1090	200 ±30	228 ±58	106 ±16	-	-	-
Camelina	19900 ±3630	21300 ±8880	473 ±83	1060 ±187	105 ±19	768 ±138	2430 ±434	138 ±25
Sunflower	17930 ±6430	348 ±187	253 ±34	-	-	-	-	-
Group IV Soybean Manhattan	44300 ±10400	7450 ±949	97 ±15	-	125 ±44	-	-	-
Group V Soybean Manhattan	40500 +/-8490	6820 ±1590	89 ±9	-	-	-	-	-
Group V Soybean Hutchinson	37900 ±10800	6070 ±1400	76 ±18	-	117 ±26	-	-	-

Values after each mean represent a 95% confidence interval

Discussion

While soaps and surfactants are the most common use of fats and oils, much work is focused on the production of more valuable specialty chemicals (Metzger and Bornscheuer, 2006). The four most widely produced chemical feedstocks from oils are free fatty acids, methyl esters or biodiesel, fatty alcohols produced from methyl esters and amines produced from fatty acids, and glycerol (Schumann and Siekmann, 2002).

Saturated oils such as palmitic acid (C16:0) and dodecanoic acid (C12:0) paired with sugar head groups have been used as surfactants in food, cosmetics, and pharmaceutical applications because of their low toxicity (Baker et al., 2000). It has been suggested that low-chain length fatty acids could be used as organic phase-change materials for latent heat storage (Kenisarin and Mahkamov, 2006 and Sarı, 2003). Monounsaturated oils such as oleic acid (C18:1) have been epoxidized and used to create polymeric formulations such as polyesters and as a drug delivery vehicle (Nicolau et al., 2010), (Luppi et al., 2005) This creates a market for value added products from oil separation.

Microbial conversion of oleic acid has been widely studied, producing products that could have applications as plasticizers, lubricants, and detergents (Hou, 1994). Oleic acid and ozone can be transformed into azelaic acid an, important intermediate for polyesters and polyamides (Baumann et al., 1988). Polyunsaturated oils such as linoleic and α -linolenic acids could be used to synthesize polyurethanes as renewable replacements for petroleum-based polymers (Keleş and Hazer, 2009).

Canola observations were close to a previous report for three lipids of interest and different for the other two. Oleic acid (18:1) was reported to have a mean of 63.5% concentration by mass in canola oil over two locations, two years, and eleven cultivars (Hamama et. al., 2003). This is 23.8% higher than the concentration of 18:1 found in the current study. Linoleic acid concentration was 11.7% higher (31.2%) than Hamama's report. Concentrations of 16:0 (4.9 reported to 3.0), 18:3 (8.1 reported to 9.4), and 20:1 (1.4 reported to 1.7) were all close to Hamama's results.

The determined lipid profile for camelina was equal parts linoleic and linolenic oil which differs from the literature greatly. Oleic and eicosenoic acids composed about 20%, or 10% each, of the lipids found while lesser amounts of both the smaller and larger lipids, most notably

palmitic acid (3.2%) and arachidic (3.2%) were found (Szterk et al., 2010). The sunflower oil profile matched the literature well, with oleic and linoleic being the most prevalent lipids found with trace amounts (<5%) of other lipids such as palmitic and stearic (Seiler et al., 2010).

Soybean profiles were compared to two articles. Kinney and Clemente, (2005) assumed soybean oil was composed of five lipids: 16:0, 18:0, 18:1, 18:2, and 18:3 and reported their concentrations as g lipid g⁻¹ total lipid. Assuming 75% of soybean oil is lipids with the remaining mass from glycerol they reported 9.8% of oil mass to be 16:0. The other four lipids considered were reported to have concentrations of 3.0%, 18:0, 13.5%, 18:1, 41.3%, 18:2, and 7.5% 18:3. These results show a 1.5 times higher concentration found for 16:0 and the same concentration found for the others. More lipids were found in the current study than these five. Although the concentration of the other unidentified lipids was low they, do not represent the entire lipid profile as more peaks were present in the gas chromatogram than could not be identified from the standard solution. Soybean bred for high oleic acid content displays higher oleic acid content stability than lines not bred for oleic acid content. Increased stability has been reported to occur when breeding for linoleic acid occurs as well (Oliva et al., 2006). Since the relative concentration of 18:1 and 18:2 are consistent with literature no evidence of breeding for 18:1 and 18:2 concentration was found.

Sunflower lipid profile was reported by Li et. al., 2011. They reported 16:0 to be 6.41%, 18:1 to be 22.97%, 18:2 to be 64.77%, and 20:1 to be 0.25%. Li's report did not find 18:3 lipids. Observed concentrations match their results for 18:1 and 18:2. The upper limit of the 95% confidence interval for 16:0 is half of Li's report. Tables 3.4 through 3.6 display the same data as tables 3.1 through 3.3 transformed into the ratio of each lipids mass to the total identified lipid mass. Production of the same cultivars in a non water stressed year would reveal if the differences in observed profiles and literature profiles are due to genotype, environment, or their interaction.

Table 3.4 Saturated fatty acids concentration for six crops grown in two locations in KS expressed as grams of lipid per gram of total identified lipid.

Crop and Location	C14	C16	C17	C18	C21	C24
	%mass	%mass	%mass	%mass	%mass	%mass
Canola Manhattan	0.550 ±0.008	2.07 ±0.297	0.166 ±0.022	30.2 ±0.172	0.842 ±0.164	0.223 ±0.040
Camelina Manhattan	0.126 ±0.051	3.22 ±0.384	-	1.13 ±0.224	-	0.155 ±0.062
Sunflower Manhattan	0.096 ±0.028	2.63 ±0.469	-	59.7 ±0.043	-	0.411 ±0.064
Group IV Soybean Manhattan	0.090 ±0.051	4.77 ±0.569	0.160 ±0.069	2.52 ±0.906	0.134 +/-0.014	0.186 ±0.171
Group V Soybean Manhattan	0.043 ±0.017	2.77 ±0.414	0.093 ±0.012	-	0.083 ±0.010	-
Group V Soybean Hutchinson	0.040 ±0.007	3.02 ±0.570	0.087 ±0.017	-	0.096 ±0.022	-

Values after each mean represent a 95% confidence interval

Table 3.5 Monounsaturated fatty acids concentration for six crops grown in two locations in KS expressed as grams of lipid per gram of total identified lipid.

Crop and Location	C16	C17	C18	C20
	%mass	%mass	%mass	%mass
Canola Manhattan	0.417 ±0.060	0.242 ±0.033	23.6 ±5.02	1.42 ±0.212
Camelina Manhattan	0.172 ±0.033	-	7.51 ±1.33	12.5 ±2.28
Sunflower Manhattan	0.307 ±0.054	-	59.7 +/-10.3	0.506 ±.102
Group IV Soybean Manhattan	0.101 ±0.017	0.106 ±0.028	15.6 ±1.90	0.230 ±0.035
Group V Soybean Manhattan	2.77 ±0.414	-	8.83 ±1.19	0.185 ±0.022
Group V Soybean Hutchinson	0.090 ±0.016	-	9.87 ±2.09	0.171 ±0.035

Values after each mean represent a 95% confidence interval

Table 3.6 Polyunsaturated fatty acids concentration for six crops grown in two locations in KS expressed as grams of lipid per gram of total identified lipid.

Crop and Location	C18:2	C18:3	C18:3n6 or C20:0	C20:2	C20:3	C20:4n6	C22:2
	%mass	%mass	%mass	%mass	%mass	%mass	%mass
Canola	23.4 ±3.77	6.73 ±1.09	0.272 ±0.041	0.307 ±0.078	-	-	-
Camelina	24.2 ±3.49	25.7 ±5.29	25.7 ±5.29	1.43 ±0.251	1.02 ±0.184	3.21 ±0.575	0.201 ±-0.037
Sunflower	21.9 ±5.48	0.421 ±0.135	0.130 ±0.017	-	-	-	-
Group IV Soybean	54.1 ±11.1	9.02 ±1.71	0.132 ±0.020	-	-	-	-
Group V Soybean	49.4 ±7.01	8.25 ±1.27	0.121 ±0.012	-	-	-	-
Manhattan							
Group V Soybean Hutchinson	46.2 ±8.89	7.35 ±1.16	0.104 ±0.024	-	-	-	-

Values after each mean represent a 95% confidence interval

Conclusions

Each crop presented unique opportunities for separation of value added lipids. Camelina had the highest collective concentration of C20:0, C16:0, and C18:3. The combination of these three lipid concentrations make camelina the most desirable oil tested for separating valuable lipids. Camelina's lipid profile is useful in production of surfactants and bioplastics. Sunflower had the highest concentration of C18:1 and canola had the highest concentration of C18:1 among the two brassicas. These concentrations present alternative markets for sunflower and canola oil, but do not outweigh the multiple occurrences of camelina having the highest concentrations of desired lipids. Linolenic acid (18:3) from sunflowers and canola offers more binding sites value added products as well. This represents the first time the lipid profile of these crops has been reviewed when grown within the same field.

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Chapter 4 - Summary

Each chapter provides an alternative perspective to bio-energy crop production. The concept of cellulosic ethanol has been around since the oil crises of the 1970's. Recent oil price surges renewed interest in cellulosic ethanol in the United States initiating the advanced biofuels initiatives of the Energy Independence and Security Act of 2007. Altering plant biomass production to favor vegetative growth increases raw materials availability for cellulosic ethanol, but harms starch crop yields. Biodiesel can be produced from several plant oil sources. Increasing cropping system intensity is a potential source of biodiesel feedstock increases. Demand for oil seed crops is an influence in the quantity of land planted in these crops. By separating higher value lipids from vegetable oil profiles an alternative market is developed for the oils. The remaining lipids after separation are then available for biodiesel production.

Population effect on cellulosic ethanol production occurred from changes in biomass totals and harvest index. Ethanol yield per unit of mass was not effect by population. In corn both the Shinozaki and Kira biomass model and the Duncan grain yield model fit the data with greater than $\alpha = 0.01$ significance in both Manhattan and Tribune. Cellulosic ethanol totals for corn were expressed at the density which produced maximum grain yield. Increasing corn density beyond maximum grain yield does provide more biomass, but the loss of grain yield along with the increased seed cost make this a poor production choice. Sorghum did not consistently respond to population. Sorghum density effects were modeled to linear, Duncan and hyperbolic (Shinozaki and Kira) effects for emerged density and end of season tiller count on grain yield, biomass yield, and stover yield for all varieties, locations, and years. Only nine of these models were significant out of 86 tested including one occurrence of two models having a significant fit on a single effect. Ethanol yields for sorghum were expressed as the highest yielding treatment for models that were significant and for the recommended seeding rate for treatments that did not have a significant model fit.

Oil production from soybean was the most reliable source of the eleven methods tested. Soybean had the highest oil yields in both locations in both years. In 2010 maturity group IV soybean yielded the most oil in both Manhattan and Hutchinson. In 2011 maturity group V soybean yielded the most oil in both locations. Maturity group IV is the better option long term as growing conditions in 2011 were atypical due to extreme water stress through much of the

growing season. Sesame produced harvestable reproductive mass in both locations in 2010 and in neither location in 2011. In 2010 camelina followed by sesame was within 15% of the maturity group IV soybean oil yield and higher than maturity group V. Further research is necessary to know if winter camelina followed by sesame would yield more oil than full season soybean. Winter camelina followed by sesame would allow longer exposure to cool season growing conditions for camelina and an earlier harvest to allow more time for sesame to mature. Sesame continues to flower until the first frost in KS under both double crop and full season cropping systems. A double crop system would need to exceed soybean oil production by more than the difference in energy used in the cropping systems to justify using a double crop system for bio-energy purposes only.

Some individual lipids are more valuable than biodiesel. Separating valuable lipids for use in bio-plastics, pharmaceutical delivery, food preservatives, cosmetics, nutritional supplements, and textiles can drive demand for oil seed production and leave the remaining lipids for biodiesel. Sunflower had the highest concentration of C18:1 and soybean had the highest concentration of C16:0 and C18:0, but camelina had the most desirable overall lipid profile. Camelina had the highest concentration of two of the lipids of interest; C18:1 and C18:3. Camelina also was the only oil to contain detectable amounts of C20:3n3/C22:0, C20:4n6 and C22:2. Although lipid profiles have been completed for each of these crops prior to this study, this represents the first time the crops' lipid profiles have been compared when grown in the same field.

Cumulatively the papers represent cellulosic ethanol production techniques from a field perspective, an attempt to increase biodiesel production beyond full season soybean feedstock production, and use of high value lipids to move biodiesel to the status of a co-product to value added products.

Appendix A - Seeding Rate Effects on Ethanol Production in Corn and Sorghum

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Abstract

Ethanol production from stover (dried stalks and leaves) results in less pressure on food supply than ethanol produced from grain. The goal of our study was to determine a relationship between seeding rate and liters of cellulosic ethanol production per hectare. Corn (*Zea mays* L.) was planted in Manhattan, Kansas (KS) (39.19° N 96.60° W) and Tribune, KS (38.28° N 101.45° W) at twice (2R), one and a half times (1.5R), the recommended (1R), half (0.5) the recommended seeding rate and without competition. A randomized complete block design was used with four replications. The grain yield and total aboveground biomass of each population was recorded. Stover preparation for fermentation began by drying and grinding to 200 µm. Three sorghum (*Sorghum bicolor* L.) cultivars were planted in Manhattan, KS and Garden City, KS (37.58° N 100.52° W) at one and a half times (1.5R), the recommended (1R) and, half (0.5R) the seeding rate. Three cultivars were used ‘M81-E sweet sorghum (Mississippi State University), ‘NK300 dual purpose forage sorghum (Sorghum Partners New Deal, Texas), Pioneer ‘84G62’ grain sorghum (Pioneer Hi-Bred, Johnson, Iowa). The grain yield was measured for the dual purpose and grain cultivars. The juice yield was measure for the sweet variety. Stover preparation began in the same manner as the corn including sweet sorghum without juicing. Cellulose, hemicelluloses, and lignin content were determined for each sample using the Natural Resource Ecology Laboratory (NREL Colorado State University interdisciplinary ecology research unit) protocol. Glucose and xylose released per gram of biomass by an acid pretreatment were different among corn populations. Baker’s yeast (*Saccharomyces cereviaceae* (E.C. Hansen) Meyen) was introduced to the biomass following saccharification. Incubation occurred at 30°C in a centrifuge at 100 rpm. Ethanol conversion

efficiencies ranged from 76.7% at 138,000 plants/hectare to 91.7% at 84,500 plants/hectare in corn. Ethanol conversion of stover mass in corn was most efficient at the recommended seeding rate. The population that produced the maximum grain yield also resulted in the maximum ethanol conversion efficiency.

Introduction

Biomass is any organic matter including, annual and perennial plants, plant fiber, and animal waste. Biomass is a renewable resource. If production and processing of biomass is completed in a responsible manner, use of it as an energy source can be sustainable. Ethanol production from hydrolysis of biomass to sugars and fermentation of those sugars is known as bioethanol. Benefits of using biomass other than plant parts used primarily for food sources include decoupling of food and bioenergy, reduction of CO₂ emissions, and insurance of a stable supply of energy (Larsen, et al., 2008)

Ethanol production from cellulosic ethanol is accomplished in a three step process. The biomass is pretreated in an H₂ SO₄ solution to break open plant cell walls. The cellulose is separated into monosaccharides by enzyme hydrolysis. The monosaccharides are fermented by baker's yeast (*Saccharomyces cerevisiae* Meyen and E.C. Hansen). Distillation is required to increase the concentration of ethyl alcohol for fuel use. The research objectives were to produce ethanol from corn stover and sweet sorghum bagasse, to determine the relationship between plant population densities and ethanol yield/ha, and to minimize the impact of ethanol production on food supply from both crops.

Historical Logistical Yield Models Used - The Duncan grain yield model was published in the Agronomy Journal in 1958 (Duncan 1958). Grain yield per plant follows an exponential decay model as population increases. The Shinozaki and Kira biomass model was published in the Journal of the Polytechnical Institute of Osaka City, Japan in 1956 (Shinozaki and Kira 1956). The end of season aboveground biomass was shown to increase hyperbolically as population increased in several crops. This model was shown to work with corn (Russell, 1979).

Both the Russell application and the Duncan grain yield model were confirmed in 2008 by Ballard (2008).

Materials and Methods

Field studies were conducted during the summer of 2009 in Manhattan, Tribune, and Garden City, KS. Corn was grown in Manhattan, KS (DKC 63-42) on a Belvue silt loam (superactive, nonacid, mesic type Udifluent) and Tribune, KS (Pioneer 33B54) on a Ulysses silt loam (superactive, mesic

Aridic Haplustolls). Sorghum was grown in Manhattan, KS and Garden City, KS on a Ulysses and Richfield complex soil. Table A.1 shows the seeding rates for each location.

Table A.1 Corn and sorghum seeding rates for each location

Seeds*ha ⁻¹	Manhattan Corn	Tribune Corn	Manhattan Sorghum	Garden City Sorghum
No Competition	2900	2900	NA	NA
0.5R	37,000	NA	74,000	37,000
Recommended (R)	64,000	29,600	144,000	74,000
1.5R	103,000	44,500	222,000	111,000
2R	140,000	59,200	NA	NA

Corn was harvested at physiological maturity by hand removing each ear in a 0.00035 ha area. The remaining stover was cut with a machete. Corn was cleaned using a stationary sheller. The grain yield was adjusted to a market moisture content of 15%. The entire stover sample was weighed as fresh weight. A subsample was dried to find the moisture content of the fresh material. Microsoft Excel was used for regression analysis of the grain yield and to demonstrate the overall biomass yields using a scatterplot. Sigma Plot will be used at a later date to further analyze the biomass results.

Stover samples were then processed into ethanol. Biomass samples were ground to a size of 200 μm . The subsample size of 15 g was mixed with a 2% solution of H_2SO_4 . The mixture was then autoclaved at 121°C for 30 minutes. Acid was removed by rinsing for one minute through a screen. This acid hydrolysis broke open the plant cell walls to allow for enzyme hydrolysis to break down the cellulose into monosaccharides. The samples were dried overnight at 60°C . Five grams of the acid hydrolysis treated biomass was mixed with 48 ml of citric acid of pH 5.0. The mixture was autoclaved at 121°C for 15 minutes.

Enzyme hydrolysis broke down the cellulose into yeast accessible six carbon sugars. The enzymes added to the biomass and citric acid were 1.25 ml of cellulase (Novozyme 22074) and 0.71 ml of glucosidase (Novozyme 50010). The enzyme hydrolysis occurred over 72 hours at 40°C in an orbital shaker at 30 rpm. The solids from the enzyme hydrolysis were separated using a centrifuge at 10,000 rpm for 20 minutes. A one ml sample of the liquid was saved for high performance liquid chromatography (HPLC) analysis. The remaining liquids were used for fermentation.

Fermentation required the input of additional nutrients and yeast. $(\text{NH}_4)_2\text{SO}_4$, 0.6 and yeast extract, 0.9 mg were added to the enzyme hydrolysis liquid. The mixture was autoclaved for 15 minutes at 121°C . After the autoclaved mixture cooled to room temperature, 3 ml of yeast broth was added. Fermentation occurred at 35°C in an orbital shaker for 15 hours. A 1 ml sample of the fermented liquid was saved for HPLC. HPLC is performed using a mixture of 10% sample and 90% deionized water.

RESULTS AND DISCUSSION

The Duncan grain yield model was statistically significant at $\alpha = 0.01$. Sigma plot analysis is yet to occur for the biomass model $M = \frac{P}{BP + A}$. Figures A.1 through A.6 show the agronomic yield results.

Preliminary bagasse ethanol yield varied from 3% to 16% by mass (July 2010). Completion of two more reps will preclude analysis of these results. Statistical analysis of these results will occur after receiving the full data tables or repeating the corn fermentation. Figures A.7 and A.8 show the sorghum bagasse yield did not vary with the population.

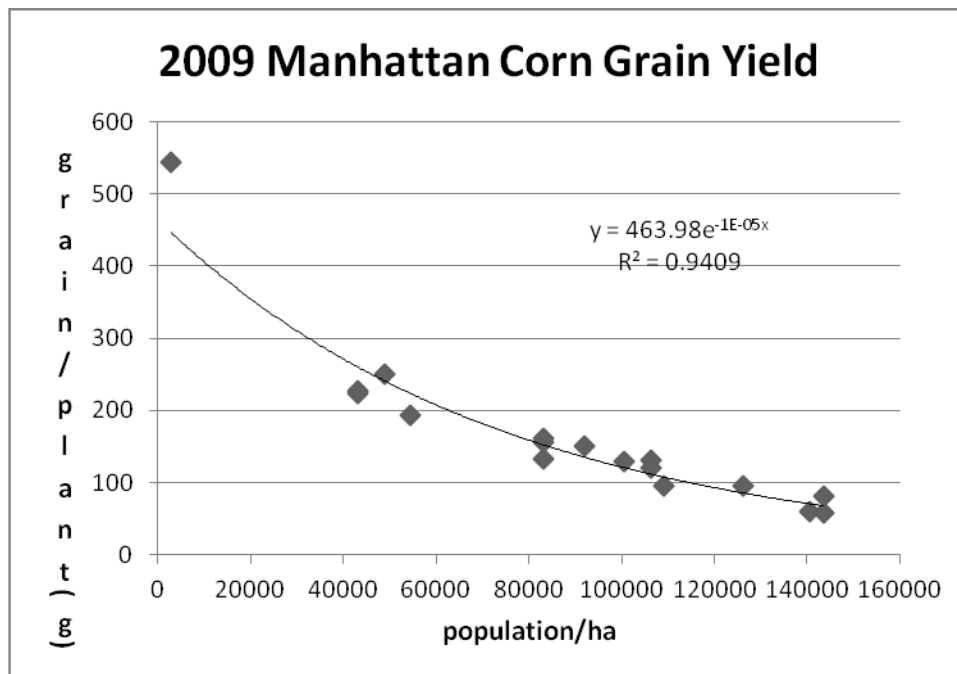


Figure A. 1 Regression Analysis of Corn Grain Yield and Plant Population at Manhattan, Kansas in 2009.

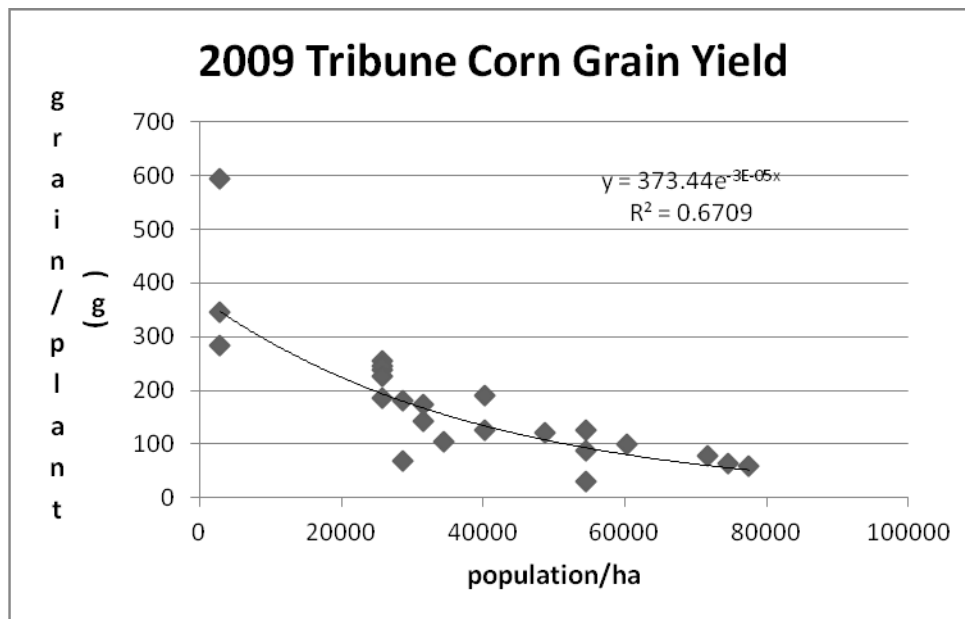


Figure A. 2 Regression Analysis of Corn Grain Yield and Plant Populations at Tribune, Kansas in 2009.

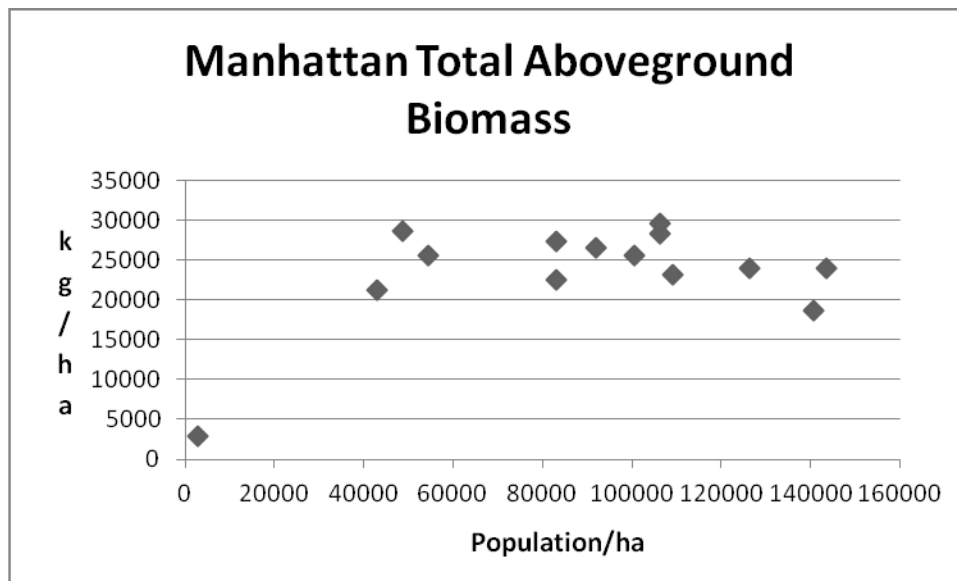


Figure A. 3 Total Corn Aboveground Biomass (Grain, Stover and Cob) as Related to Plant Populations at Manhattan, Kansas in 2009

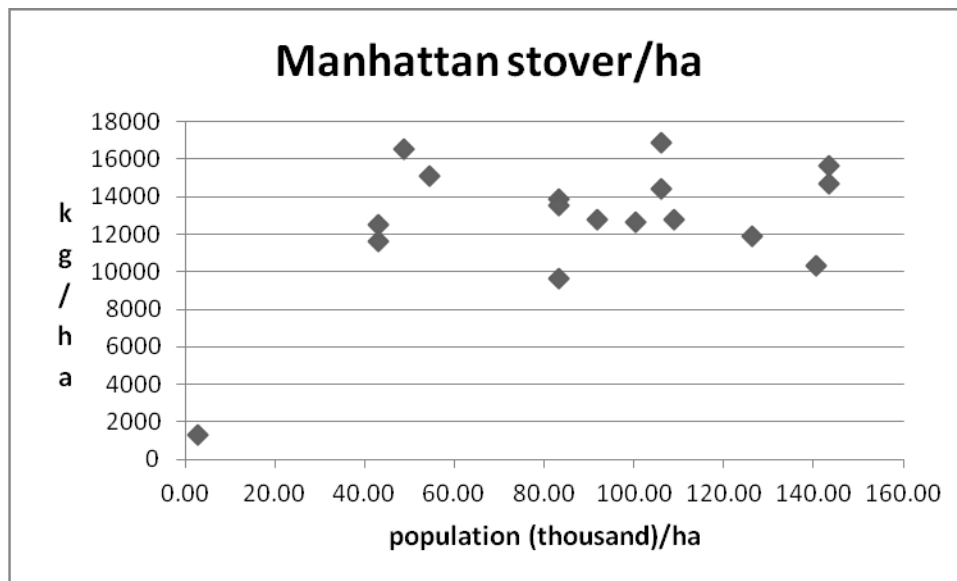


Figure A. 4 Stover Biomass as Related to Plant Poplations at Manhattan, Kansas in 2009.

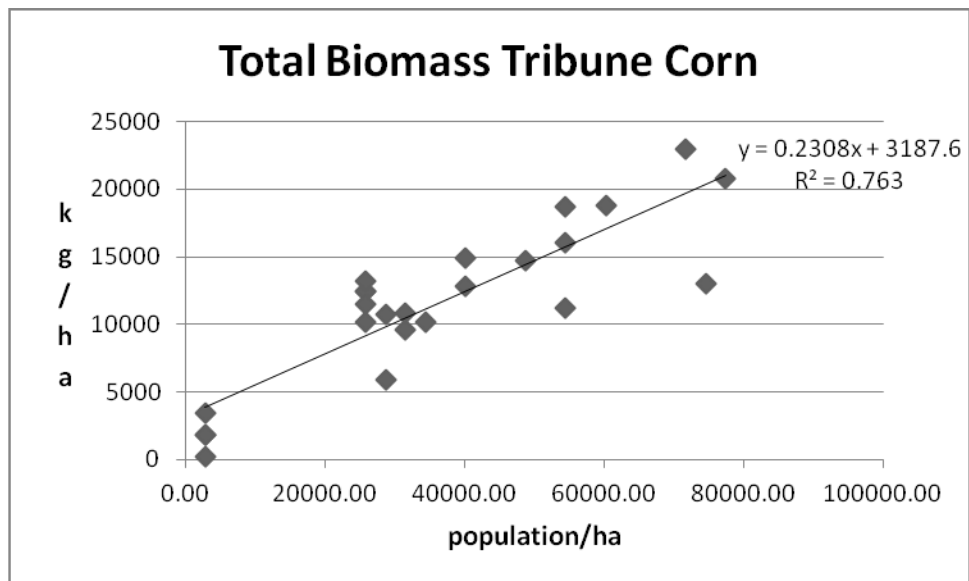


Figure A. 5 Regression Analysis of Total Corn Biomass (Grain, Stover, and Cob) and Plant Populations at Tribune, Kansas in 2009

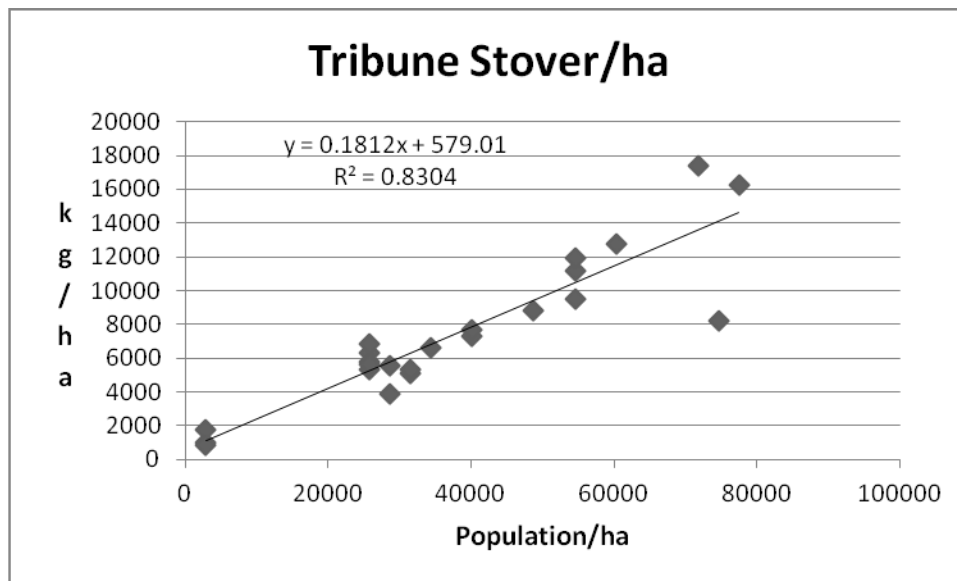


Figure A. 6 Regression Analysis of Corn Stover Biomass and Plant Populations at Tribune, Kansas in 2009.

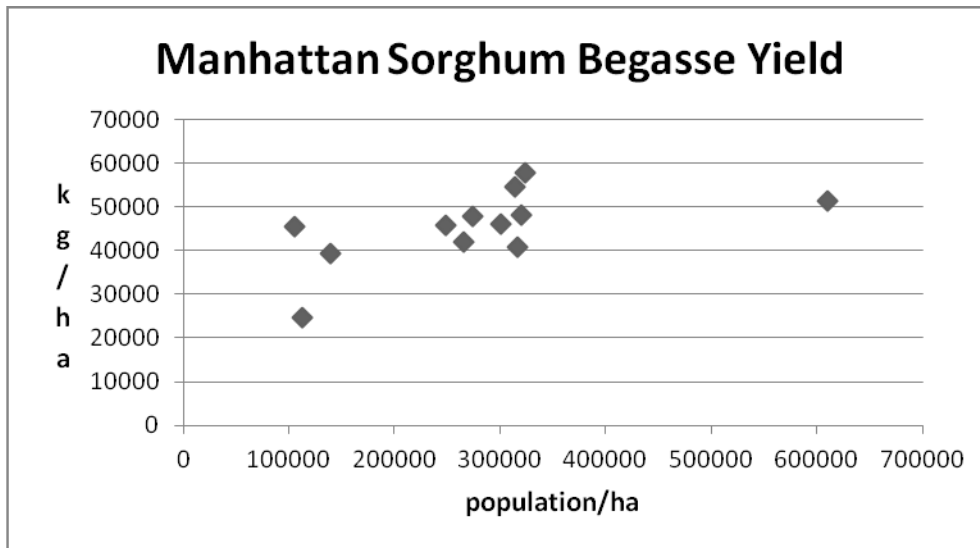


Figure A. 7 Sorghum Biomass Yield (dried sweet sorghum stover after juice (50% of sucrose) was extracted) as Related to Plant Populations at Manhattan, Kansas 2009

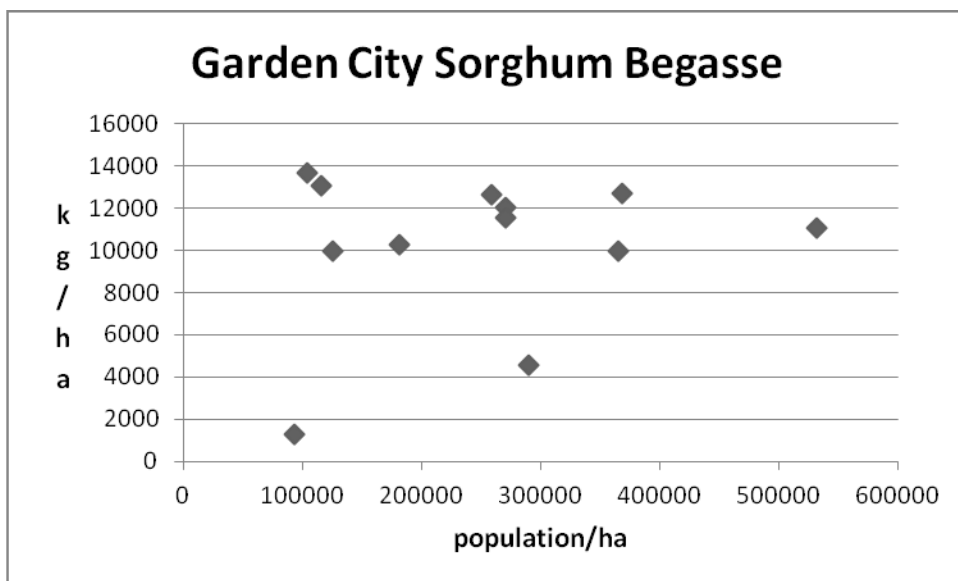


Figure A. 8 Sorghum Biomass Yield (dried sweet sorghum stover after juice (50% of sucrose) was extracted) as Related to Plant Populations at Tribune, Kansas 2009

CONCLUSIONS

A model is being developed to explain the differences in bagasse ethanol yield. Corn stover ethanol yield appears steady. If the differences shown in preliminary analysis are statistically significant, the differences would be from differences in the relative proportion of rind to pulp occurring at different populations. Combining the stover masses and the cellulosic ethanol percentage by mass reveal the net ethanol*ha⁻¹. Minimal changes in grain yield over a wide range of corn populations allow for adjusting the population to increase cellulosic ethanol yield. Ethanol production from the remaining sorghum stovers is currently being processed.

References for Appendix A

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